Ninette Jones

History reveals that before the construction of the Bonneville Dam that there have always been bottlenecks on the Columbia River where fish would pool and rest and humans and marine mammals could eat them. Historians note log jams, beaver dams, and the great Celilo Falls is once where the strongest and fastest salmon escaped dip nets and hungry animals from winter until the spring as Chinook salmon ascended the tumultuous river & thunderous & sacred falls. One Hundred and thirty other non human animal species rely on salmon for their very sustenance --not sport. In the Columbia River estuary sea lions and seals could easily feed on fish through the winter until the spring as Mother Nature intended, and there were always enough salmon for the tribal fisheries, abundant populations of bears (grizzly and black), wolves, coyotes, bobcats, lynx, osprey, terns, loons, herons, eagles, mergansers, American dippers, cormorants and on and on that all subsisted in part on differing life histories of salmon and steelhead within the Columbia Basin(B. Mc Millan 2008). Celilo Falls although, created an impassable bottleneck for pinnipeds on the Columbia River.

Moreover, healthy salmon swim faster than sea lions by Mother Nature's design and as history has shown us the Columbia River was once a series of tumultuous swirling, frothy, cold, rolling rapids; rushing to propel, young salmon, down river on their outward migration, towards their adult habitat, the sea. The Columbia River habitat now consists of warming narrow channels and slack water lakes created by the US ACOE hydroelectric dam's reservoirs and now Chinook salmon returning to Idaho's Snake River must pass eight dams twice in their lifetime. National Marine Fisheries now reports a 20% conversion rate for these salmon at each passage facility so the US ACOE providing intentional adequate river flow over the dam in the spring will help push young salmon towards the sea in a timely manner.

Altogether, cold water spilling over the dam helps young fish avoid the dam's turbines, avoid lenthic warming aquatic habitat conditions that now favors non- native piscivorious fish such as small-mouth bass, walleye, channel catfish, northern pike, pike minnow and American shad (NMFS, Sanderson 08) over native cold water fish. Most of the non- native fish- eating fish were and are still intentionally stocked or not, and or released into the Columbia River for sport fishing. And these non native and hatchery fish populations are all well known for competing with the salmon for food and habitat resources and non-native and hatchery fish known for predating heavily on millions and millions of baby salmon as they float down river on their outward migration towards their adult habitat the sea. Non- native fish populations now make up the most abundant populations of fish in the Columbia River estuary.

On the other hand what we know about marine mammals is that sea otters, Steller sea lions and southern resident orca populations are all important native key- stone species in the Pacific Northwest bioregion. Sea lions for example are opportunistic eaters and they are breast stroke swimmers and sea lions tend to consume the prey that is most abundant in the estuaries which are now populations of non native and hatchery fish (Sanderson08). The US ACOE observers at the dam have reported that not all sea lions that visit the Bonneville Dam are proficient at catching salmon. In addition, according to sea lion scat samples taken in the lower estuary-- NMFS reports that 90% of the time sea lions diets do not consist of salmon. Sea lions and many other species of marine mammals and Chinook salmon have always called the Columbia River estuary home and

both of these species thrived and survived together in huge populations just fine for over ten thousand years in the Columbia River estuary.

In addition, sea lions and other species of marine mammals all have very important jobs to perform in our Pacific Northwest ecology. Attached is a peer reviewed study that compares the difference between human and the sea lion's gut flora and highlights these important differences to show how the sea lion's gut flora are corner stone in the food web for all life in the oceans. Altogether, sea lions have 60 plus micro- biomes that are significantly different than the human micro-biomes and that sea lions and whales are essential nutrient productivity pumps that enhance the health of our rivers, oceans and estuaries. It is now, known, how important top native, non- human animal predators are such as, whales, orcas, wolves, sea otters and steller sea lions that all have the power to potentially influence change across terra and aquatic landscapes down to the plant life, influence the climate, influence the health & distribution of prey, and that removing key stone species can directly influence a river's flow. Native key stone species are very valuable for the Columbia River estuary and losing them will be a great loss for many populations of fish species

In the end the state of Oregon and Washington waging war on sea lions below the Bonneville Dam and on a Superfund site called the Willamette River undermines the productivity of the food- web in the Columbia River estuary and her tributaries; it does not enhance it (trophic cascades). Top key stone species like steller sea lions, sea otters and southern resident orcas keep the health of the Pacific Northwest ecology in check by predating on the weak, the sick, the old, the injured, NIS and hatchery fish. Steller sea lions and southern resident orca are both important species in providing food for scavengers and for promoting estuary health -- the sea lion's gut flora creates fish food and these animals bring life enhancing nutrients into the estuary and up river. The presence and protection of many populations of marine mammal species in the Columbia River estuary is corner stone in protecting and enhancing the productivity of the food web, and important for strengthening the hearts, minds and strengthening the very genetics of the native wild cold water fish. As well, the US ACOE intentionally, providing adequate spill of cold flowing river water over their eight dams in the spring is a positive step towards ensuring the survival for many, many human and non-human animal species in the Columbia River estuary for many future generations to come.

For the children, marine mammals, salmon, and the Columbia River estuary.

Ninette Jones

Portland, Oregon

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1. http://bioscience.oxfordjournals.org/content/59/3/245.full

2. http://bioscience.oxfordjournals.org/content/59/3/245.full

3. "The volume of plant plankton has declined across much of the world over the past century, probably as a result of rising global temperatures. But the decline appears to have been been steepest where whales and seals have been most heavily hunted. The fishermen who have insisted that predators such as seals should be killed might have been reducing, not enhancing, their catch".

https://www.newscientist.com/article/mg21128201-700-vital-giants-why-living-seas-need-whales/

4. Lichatowich, Jim. "Salmon, People and Place " A Biologist's Search for Salmon Recovery. Corvallis OR: Oregon State University Press, 2013.

5. https://lewisandclarkjournals.unl.edu/search?utf8=&qfie ld=text&qtext=phoca rock

6. https://lewisandclarkjournals.unl.edu/item/lc.jrn.1806-02-23

7. B. Mc Millan 2008 Researching Columbia Sea lion Population

High Nutrient Transport and Cycling Potential Revealed in the Microbial Metagenome of Australian Sea Lion (*Neophoca cinerea*) Faeces

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Abstract

Metagenomic analysis was used to examine the taxonomic diversity and metabolic potential of an Australian sea lion (*Neophoca cinerea*) gut microbiome. Bacteria comprised 98% of classifiable sequences and of these matches to *Firmicutes* (80%) were dominant, with *Proteobacteria* and *Actinobacteria* representing 8% and 2% of matches respectively. The relative proportion of *Firmicutes* (80%) to *Bacteriodetes* (2%) is similar to that in previous studies of obese humans and obese mice, suggesting the gut microbiome may confer a predisposition towards the excess body fat that is needed for thermoregulation within the cold oceanic habitats foraged by Australian sea lions. Core metabolic functions, including carbohydrate utilisation (14%), protein metabolism (9%) and DNA metabolism (7%) dominated the metagenome, but in comparison to human and fish gut microbiomes there was a significantly higher proportion of genes involved in phosphorus metabolism (2.4%) and iron scavenging mechanisms (1%). When sea lions defecate at sea, the relatively high nutrient metabolism potential of bacteria in their faeces may accelerate the dissolution of nutrients from faecal particles, enhancing their persistence in the euphotic zone where they are available to stimulate marine production.

Introduction

Mammalian body surfaces are colonised by microbial communities that often exist in a mutualistic relationship with their mammalian host [1]. Mutualistic interactions between the gut microbiota and mammalian hosts have evolved over a long co-evolutionary process [2]. The microbial community of an organism is termed the 'microbiome' and the gastrointestinal microbiome has a crucial role in gut physiology, defence against pathogens, maturity of the immune system and the recovery of metabolic energy for the host [3]. The gut microbiome synthesises vitamins and amino acids and aids in the breakdown of otherwise indigestible foods [1].

Gut microbes have previously been examined by isolating and sequencing bacterial species from faeces [3]. However, the advent of metagenomic techniques has allowed for a more comprehensive and unbiased assessment of microbial genomic diversity within the complex gut ecosystem by allowing for examination of organisms not easily cultured in a laboratory [4]. Metagenomic analysis of faeces allows for characterisation of the microbial community within the gut [1] and can elucidate important processes for the gut microbes and the host and provide insight into links between the host, gut microbes and the surrounding ecosystem [1,3–5].

Here we characterise the community composition of an Australian sea lion faecal microbiome and compare the metabolic potential with other microbiomes. In doing so, we provide the first information on the gut microbiome of an Australian sea lion. We examine a marine mammal specifically, in light of recent research highlighting the role of marine mammal faeces in the nutrient cycle of the ocean [6]. We consider whether bacteria might enhance the persistence of Australian sea lion faecal nutrients in the photic zone by solubilising nutrients from the faecal particles before the faecal particles can sink to the deep ocean.

Methods

Sample Collection

Australian sea lions (*Neophoca cinerea*) number approximately 11 000 with the major population occurring in South Australia [7]. Australian sea lions predominantly consume squid and fish prey and dive to average depths of roughly 40–80 m while foraging [8]. A faecal sample from an Australian sea lion was collected from Seal Bay, Kangaroo Island, South Australia (35°59.842′S, 137°19.484′E). The sample was collected within 20 minutes of defecation using a sterile scalpel and care was taken to ensure that sampling did not include any faeces in direct contact with the ground or contaminated by seawater. The sample was placed in sterile 50 ml plastic tubes and retained on ice at approximately 4°C for <12 hours during transport. The sample was then frozen at −80°C.

Metagenomic Sequencing

Microbial community DNA was extracted from 30 grams of faeces using a bead beating and chemical lysis extraction kit

(MoBio, Solano Beach, CA.) and further concentrated using ethanol precipitation. DNA quality and concentration was determined by agarose gel electrophoresis and a nanodrop spectrophotometer respectively. Over 6 µg of high molecular weight DNA was sequenced using a 454 GS FLX (Roche) pyrosequencing platform at the Australian Genome Research Facility.

Data Analysis

Unassembled sequences were annotated using the MetaGenomics Rapid Annotation using Subsystem Technology (MG-RAST) pipeline version 2.0 (http://metagenomics.nmpdr.org/) [9]. The MG-RAST pipeline implements the automated BLASTX annotation of metagenomic sequencing reads against the SEED non-redundant database [10], a manually curated collection of genome project-derived genes grouped into specific metabolic processes termed 'subsystems'. The SEED matches of Protein Encoding Groups (PEGs) derived from the sampled metagenome may be reconstructed in terms of either metabolic function of taxonomic identity at varying hierarchical levels of organisation. The MG-RAST pipeline was used to perform quality control on the sequences by removing reads with greater than 10 ambiguous bases per read and dereplicating artificial duplicates in which the first 50 bp of the read were identical. Phylogeny was assigned by matching sequences to the SEED database [10] using BLASTX with an e-value of 10^{-5} and a minimum alignment length of 50 bp. Similarly, sequence reads were assigned to metabolic subsystem pathways using MG-RAST and a BLASTX e-value cut-off of 10^{-5}.

The metabolic potential of the Australian sea lion faecal microbiome was compared to metagenomes sequenced from other faecal samples, seawater samples and whale fall samples publicly available on the MG-RAST server using PRIMER. Relative proportions of metabolic subsystem categories were generated using the heatmap function in MG-RAST before being exported to PRIMER. Relative proportions were normalised by sequence matches to control for sequencing effort before being square root transformed. Bray Curtis similarity was used to construct a Multi-Dimensional Scaling plot. The MDS was used to determine the sample that most closely clustered to the metabolic potential of the Australian sea lion faecal microbiome. The STatistical Analysis of Metabolic Profiles (STAMP) package [11] was used conduct a Fisher's exact test with the Storey's FDR correction applied in order to conduct a fine scale examination of differences in metabolic potential between the Australian sea lion faecal microbiome and the most similar sample. Corrected P-values (q-values) were calculated with those that were <0.05 being deemed significant. The corrected p-value indicates the expected proportion of false positives within the set of features with a smaller q-value. A Fisher's exact test was also carried out between the Australian sea lion faecal microbiome and a healthy fish gut microbiome to elucidate differences between organisms that share a similar environment. We then considered gene sequences that are over-represented in the Australian sea lion faecal microbiome compared to both the most similar metagenome and the fish gut microbiome and gene sequences that are over-represented in the Australian sea lion faecal microbiome compared to two Antarctic seawater samples. To facilitate comparison between metagenomes with smaller read lengths no minimum base pair alignment length was set when comparing microbiomes. The Australian sea lion faecal microbiome is publically available on the MG RAST pipeline (http://metagenomics.nmpdr.org/, MG RAST ID: 4446343.3).

Results

Australian Sea Lion Faecal Bacteria Taxonomy

Whole community microbial DNA from a fresh sample of Australian sea lion faeces was sequenced and yielded 45 760 contigs totalling 14 124 226 base pairs with an average fragment length of 309. A total of 20 843 sequences (45.55%) could be matched to proteins in SEED subsystems. Of these, 98% of similarities were to bacterial, 1.38% to archaea, 0.46% to eukaryota, 0.17% to viruses and 0.01% were to plasmids. Our data represents the most abundant members of the community which are thriving in the current ecological conditions and does not address the 'rare biosphere' of low abundance taxa. This is an inherent feature of all metagenomic studies and is adequate when inferring metabolic potential because a large amount of biogeochemical cycling is carried out by the most abundant community members.

Bacterial phylogenetic diversity was dominated by *Firmicutes* (80% of bacterial sequences), *Proteobacteria* (8% of bacterial sequences) and *Actinobacteria* (2% of bacterial sequences) (Figure 1A). *Firmicutes* were dominated by *Clostridia* (77% of *Firmicutes*) and *Bacilli* (21% of *Firmicutes*) (Figure 1B). *Proteobacteria* were dominated by *Gammaproteobacteria* (49% of *Proteobacteria*) and Alphaproteobacteria (13% of Proteobacteria) (data not shown).

Australian Sea Lion Faecal Bacteria Metabolic Analyses

The metabolic potential of the Australian sea lion faecal microbiome was dominated by a clustering based subsystem (14%) and genes coding for core metabolic functions such as carbohydrate utilisation (14%), protein metabolism (10%) and DNA metabolism (7%) (Figure 2A). The clustering based subsystem was in turn made up of a clustering subsystem category (43%) which included putative hemin transporters and bacterial RNA metabolizing Zn dependent hydrolases (data not shown), 6% cell division and 6% protein export (Figure 2B). Carbohydrate utilisation (Figure 2A) was made up of 33% clustering based subsystems, 20% di- and oligosaccharides and 15% central carbohydrate metabolism (Figure 2C).

Comparison of Australian Sea Lion Faecal Microbiome with other Faecal, Seawater and Whale Fall Microbiomes

The metabolic potential of the Australian sea lion faecal microbiome was compared to 21 microbiomes publicly available on the MG-RAST server. The compared microbiomes comprised of seawater samples (Antarctic, North Pacific, South Pacific and Indian Oceans), gut microbiomes (human, fish, cow and chicken), and whale falls (Table 1). The Australian sea lion faecal microbiome clustered most closely with human gut microbiomes, with avian and cattle gut microbiomes also clustering near the sea lion faecal microbiome (Figure 3).

The human gut microbiome (termed Human A) most similar to the sea lion faecal microbiome was used for a finer scale examination of the differences in taxonomic and metabolic potential between the two samples. Statistical analyses revealed a total of 23 significant differences in taxonomic diversity between the Australian sea lion faecal microbiome and the Human A faecal microbiome (Figure S1). The Australian sea lion faecal microbiome was over-represented in *Firmicutes* and under-represented in *Bacteroidetes* compared to the Human A faecal microbiome. There were 63 significant differences in metabolic potential between the Human A and Australian sea lion microbiomes (Figure S2). The sea lion microbiome was over-represented in comparison to Human A microbiome in regard to 28 functions and pathways including electron accepting reactions, protein biosynthesis, ABC
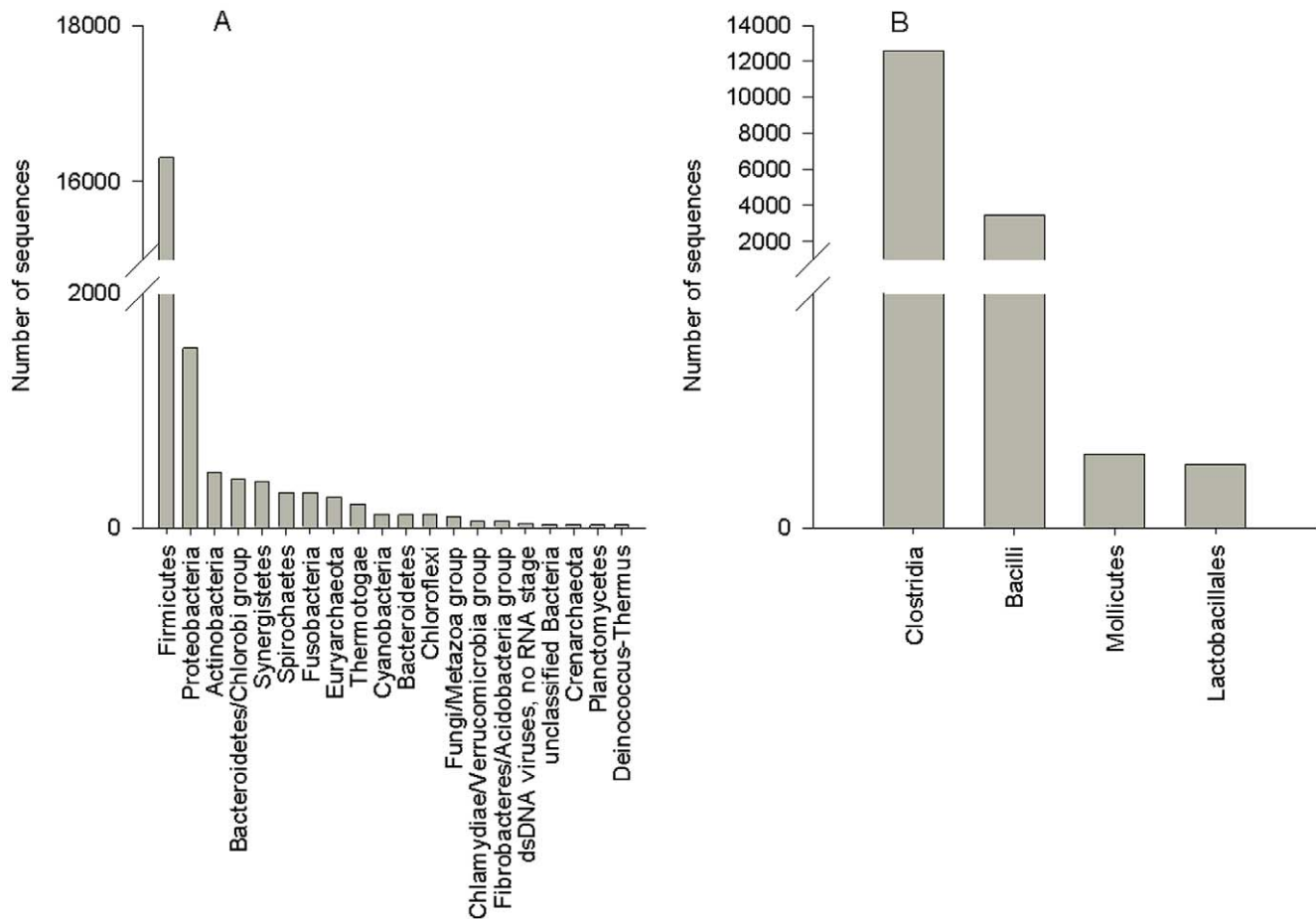
Figure 1. Taxonomic Diversity of Australian Sea Lion Gut Microbiome. A: The Australian sea lion gut microbiome was dominated by *Firmicutes* and *Proteobacteria*. The following phyla were also present in the ASL gut microbiome but had <10 sequences and thus are not shown on the graph: *Aquificae*, *Viridiplantae*, *Korarchaeota*, Bacteriophage ROSA, *Englenozoa*, *Lactobacillus plantarum* bacteriophage phiJL-1, Plasmid PCD4, Plasmid pIP404, Environmental samples, ssRNA negative strand viruses. B: *Firmicutes* were in turn dominated by *Clostridia* and *Bacilli*.
doi:10.1371/journal.pone.0036478.g001

transporters, phosphorus metabolism and iron scavenging mechanisms.

As a mammal that forages exclusively in the ocean, sea lions have a distinctive life history. Therefore, we also examined differences in the taxonomic and metabolic potential within the context of an ocean habitat, i.e., between the Australian sea lion faecal microbiome and an aquacultured fish (Fish A) gut microbiome. There was greater dissimilarity between the Australian sea lion faecal microbiomes and the fish faecal microbiome than was observed between the sea lion and human gut microbiomes (Figure 3). Fisher's exact test revealed 35 significant differences in phyla between the Australian sea lion faecal microbiome and the Fish A faecal microbiome (Figure S3). The Australian sea lion faecal microbiome was over-represented in genes coding for *Firmicutes* and under-represented in genes coding for *Proteobacteria*. In regard to metabolic potential, the Australian sea lion microbiome was over-represented in comparison to Fish A microbiome in regard to 57 pathways and functions (Figure S4) including di- and oligosaccharides, cell cycle in prokaryota, DNA metabolism, membrane transport, protein biosynthesis, iron scavenging mechanisms and phosphorus metabolism. An analysis of the comparisons between the Australian sea lion microbiome and the human and fish microbiomes, reveals 19 metabolic processes in which the sea lion faecal microbiome is significantly enriched in comparison to both Human A and Fish A microbiomes (Figure 4A) and 18 processes in

which both the Human A and Fish A microbiomes are significant enriched in comparison to the Australian sea lion faecal microbiome (Figure 4B).

To further examine differences in metabolic potential within the context of an ocean habitat, we compared the metabolic potential of the Australian sea lion faecal microbiome to two Antarctic seawater microbiomes (termed Antarctic seawater A and Antarctic seawater B). Fisher's exact test revealed 28 significant differences in metabolic potential between the Australian sea lion microbiome and Antarctic seawater sample A (Figure S5) and 27 significant differences in metabolic potential between the Australian sea lion faecal microbiome and Antarctic seawater sample B (Figure S6). There were 16 metabolic processes that were over-represented in the Australian sea lion faecal microbiome compared to both Antarctic seawater samples (Figure 5A) and 11 metabolic processes that were under-represented in the Australian sea lion faecal microbiome compared to both Antarctic seawater samples (Figure 5B).

Discussion

Australian Sea Lion Gut Microbiome Taxonomy

Our findings indicate that the Australian sea lion gut microbiome is dominated by the same four bacterial phyla that dominate the human gut (*Firmicutes*, *Proteobacteria*, *Bacteroidetes*,
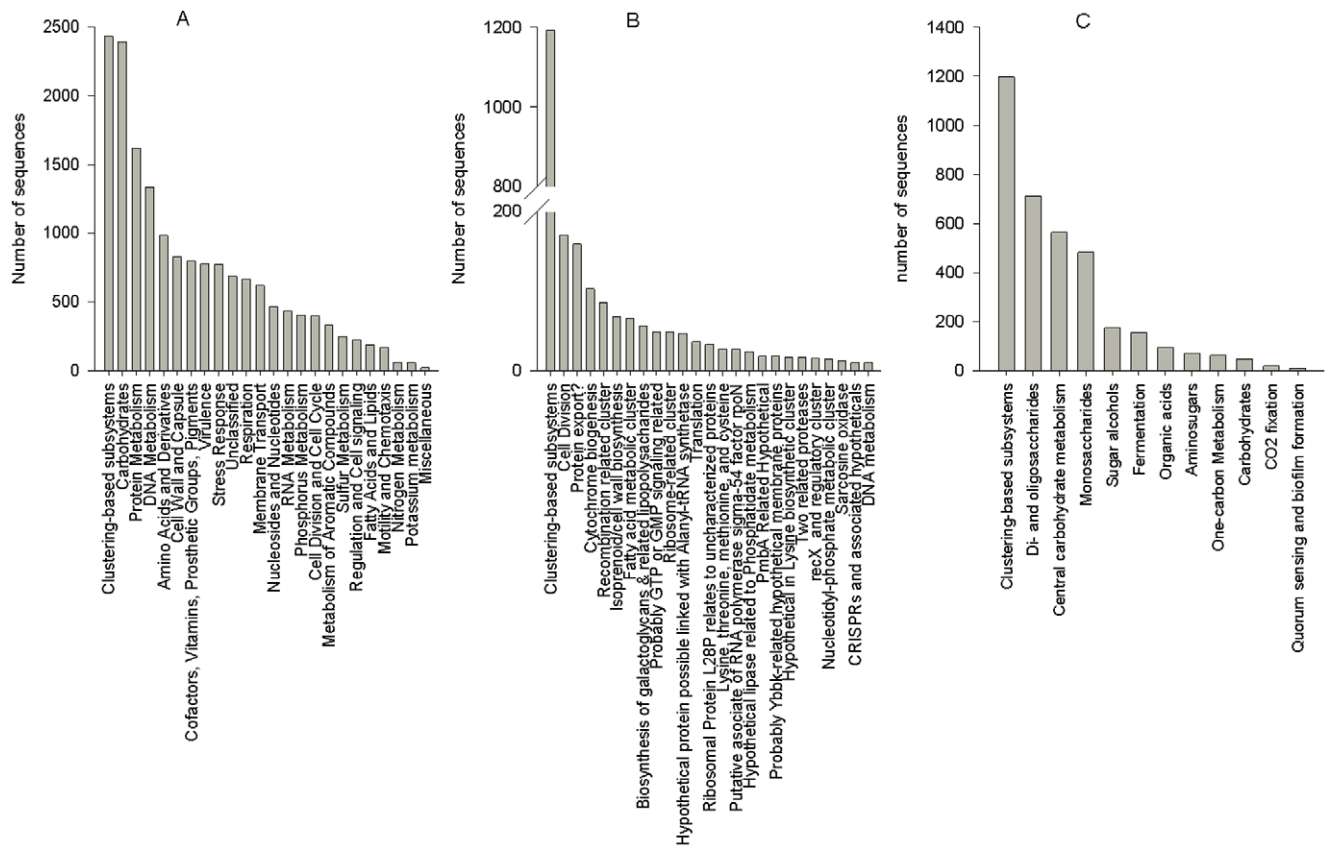
Figure 2. Metabolic Potential of Australian Sea Lion Gut Microbiome. A: The metabolic potential of the Australian sea lion gut microbiome is dominated by clustering-based subsystems and carbohydrates. Protein metabolism and DNA metabolism are also highly represented. Sequences coding for prophage, secondary metabolism, macromolecular synthesis and dormancy and sporulation were also present but were represented by <10 sequences each and hence are not shown here. B: The metabolic potential of the clustering based subsystems in the Australian sea lion gut microbiome are dominated by clustering based subsystems, cell division and protein export. The following metabolic functions were also present but had <10 sequences and are not shown here: hypothetical associated with RecF, carotenoid biosynthesis, tricarboxylate transporter, probably organic hydroperoxide resistance related hypothetical, protein, pigment biosynthesis, related to N-acetylglucosamine utilization subsystem, TldD cluster, tRNA sulfuration, chemotaxis, response regulators, cluster of unknown function, DNA polymerase III episolon cluster, lipoprotein B cluster, putrescine/GABA utilization cluster, D-tyrosyl-tRNA (Tyr) deacylase (EC'3.1.-.-) cluster, metaylamine utilisation, putative GGDEF doman protein related to agglutinin secretion, and siderophore biosynthesis. C: The clustering-based subsystems were further dominated by clustering-based systems (hierarchical level 3), di- and oligosaccharides, central carbohydrate metabolism, monosaccharides.
doi:10.1371/journal.pone.0036478.g002

Actinobacteria) [12]. Compared to both Human A and Fish A faecal microbiomes, the Australian sea lion microbiome was over-represented in *Firmicutes* (Figures S1 and S3). In humans and mice, the relative proportion of *Firmicutes* to *Bacteriodetes* has been found to be a factor in obesity, with obese humans and mice having relatively fewer *Bacteriodetes* and more *Firmicutes* compared to lean subjects [13–15]. In the Australian sea lion faecal microbiome, the percentage of *Firmicutes* (80% of total sequences) to *Bacteriodetes* (2%) is similar to the relative proportions in obese mice and human subjects [13]. While many factors, such as diet and physiology, may influence body mass, the faecal microbiome of the Australian sea lion may confer a predisposition towards excess body fat. Excess body fat is an advantage for an endothermic mammal such as a sea lion that must maintain a stable, high (36 to 38°C) body temperature despite living in a fluid in which heat is conducted away from the body at 25 times faster than in air [16].

Australian Sea Lion Microbiome Metabolic Potential

As in other gut microbiomes core metabolic functions including carbohydrate and protein metabolism dominated the Australian sea lion gut microbiome [5]. Carbohydrates serve an important

role in energy storage within the gut. Protein metabolism is also a core function of the gut microbiome. While most microorganisms and plants can biosynthesise amino acids, animals must consume proteins as part of their diet in order to gain the amino acids needed for cell functioning. There were 63 significant differences in metabolic potential between Australian sea lion and Human A microbiomes (Figure S2) and 110 significant differences observed between the Australian sea lion and Fish A microbiomes (Figure S4). Overall, 19 metabolic processes were significantly enriched in the Australian sea lion microbiome compared to both the Human A and Fish A microbiomes (Figure 4A).

The Australian sea lion gut microbiome had significantly enriched numbers of genes coding for protein biosynthesis and membrane transport. Membrane transport genes are often overrepresented in gut microbiomes [17]. Diets high in fish have high levels of purines [18] and the high purine levels in the exclusive fish and cephalopod diet of Australian sea lions [19] may provide the resources for the observed over-representation of genes associated with DNA replication, DNA repair and cell division in the sea lion gut microbiome. Selenoproteins were also enhanced in the Australian sea lion microbiome compared to

Table 1. Publically Available Metagenomes used for Comparison with the Australian Sea Lion Gut Microbiome. Number of hits determined with BLASTX E value of 10^{-5}, no minimum base pair alignment length.

Title	MG-RAST ID	Description	Number of hits (phylogeny)	Number of hits (metabolism)
Sea lion	4446343.3	Australian sea lion faeces	24297	16804
Human(A)	4440946.3	Human faeces - Kurokawa human In-A	16743	11967
Human(B)	4440945.3	Human faeces - Kurokawa human In-B	8801	5306
Human (C)	4440940.3	Human faeces – Kurokawa human F1-U	14896	12275
Cow(A)	4441679.3	Cow rumen –640F6	24443	16189
Cow(B)	4441682.3	Cow rumen – pooled plankton	24600	15745
Cow (C)	4448367.3	Cattle faecal pool	156192	100945
Fish(A)	4441695.3	Fish – Healthy gut bacteria	12453	7544
Fish(B)	4441696.3	Fish – morbid gut bacteria	13307	8086
Fish (C)	4440066.3	Aquacultured fish	11667	7405
Fish (D)	4440065.3	Aquacultured fish	5237	3263
Chicken	4440283.3	Chicken cecum	54877	30674
Antarctic (A)	4443686.3	Antarctica Aquatic Microbial Metagenome_8	92148	69892
Antarctic (B)	4443687.3	Antarctica Aquatic Microbial Metagenome_9	89222	68848
Arctic (A)	4440306.3	Arctic seawater	81674	52807
Arctic (B)	4441622.3	Arctic seawater – Chukchi	135541	75370
Xmas (A)	4440038.3	Northern Line Islands	45741	33654
Xmas (B)	4440041.3	Northern Line Islands	5484	2740
ALOHA	4441057.4	HOT/ALOHA upper euphotic	6590	4426
Whale fall	4441619.3	Whale fall bone	36057	25884
Whale fall	4441656.4	Whale fall mat	32133	23177
Whale fall	4441620.3	Whale fall rib	34525	26119

doi:10.1371/journal.pone.0036478.t001
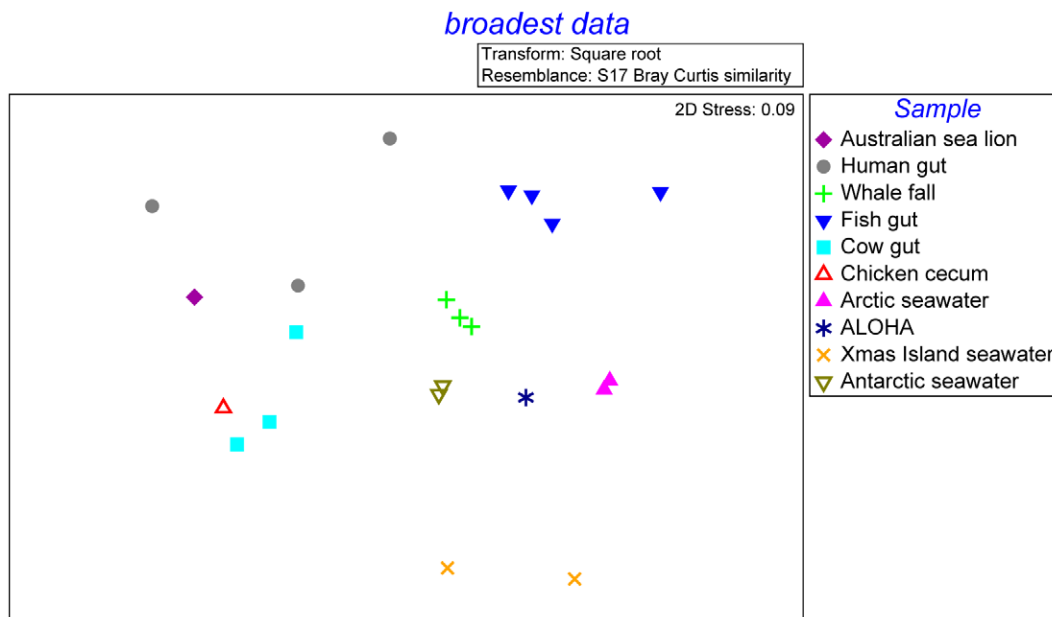
Figure 3. Multi-Dimensional Scaling Plot Comparing Australian Sea Lion Microbiome Metabolic Potential with several other Gut, Seawater and Whale Fall Microbiomes. Metabolic potential of the Australian sea lion gut microbiome is compared to publicly available seawater samples (Antarctic, North Pacific, South Pacific and Indian Oceans), gut microbiomes (human, fish, cow and chicken), and whale fall microbiomes from the MG-RAST server.
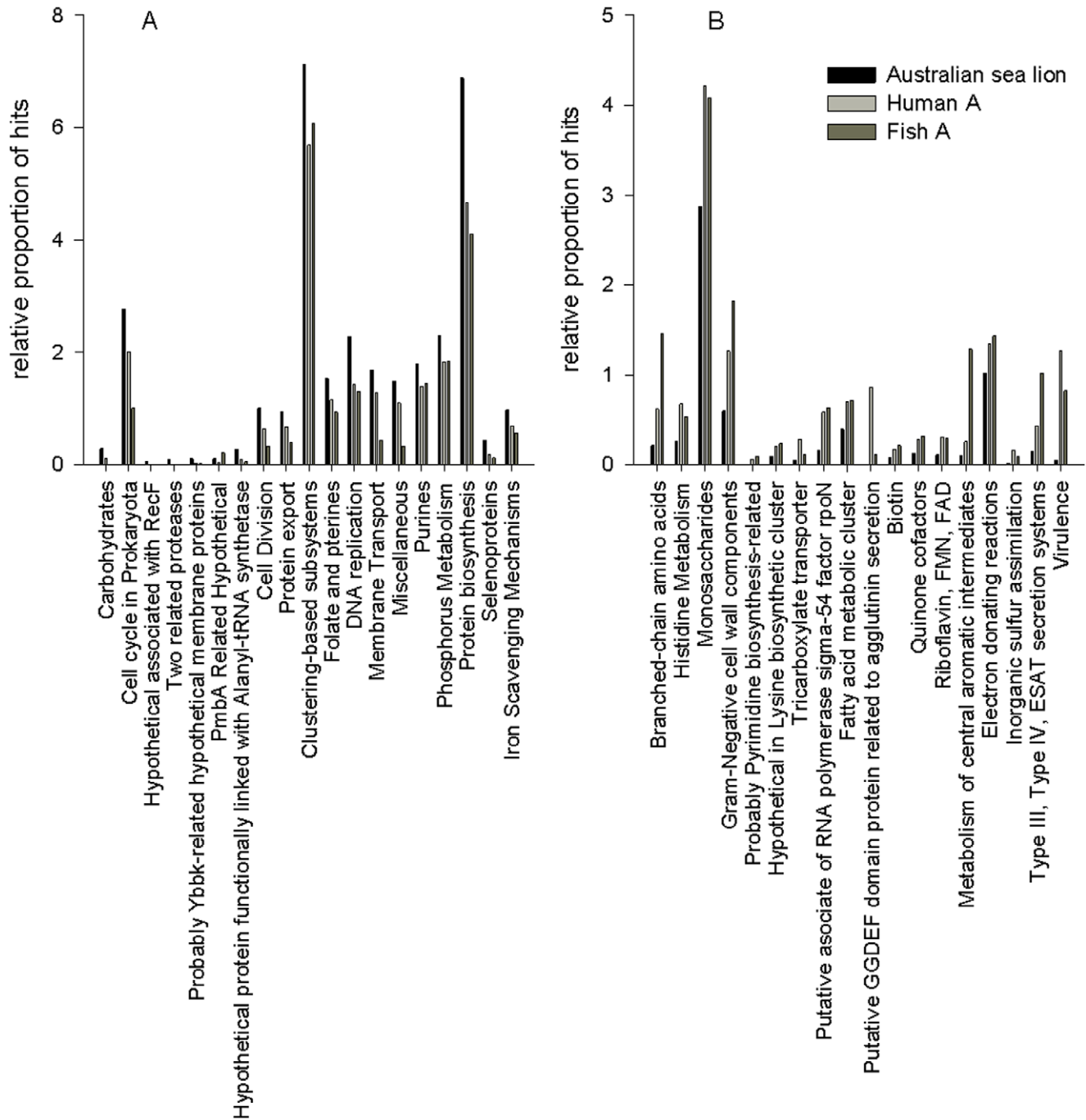doi:10.1371/journal.pone.0036478.g003

Figure 4. Metabolic Subsystems Over-represented and Under-represented in the Australian Sea Lion Faecal Microbiome compared to both Human A and Fish A Gut Microbiomes. A: The metabolic subsystems that are over-represented in the Australian sea lion faecal microbiome compared to Human A and Fish A gut microbiomes. B: The metabolic subsystems that are under-represented in the Australian sea lion faecal microbiome compared to Human A and Fish A gut microbiomes.
doi:10.1371/journal.pone.0036478.g004

Human A and Fish A microbiomes which may suggest that the Australian sea lion gut is nutrient deficient relative to other gut microbiomes. Selenoproteins are involved in glycine reductase activity which incorporates the use of dithiol to reduce glycine to acetate and ammonia [20]. Glycine reductase activity is increased when *Clostridia* are grown in nutrient deficient conditions [21].

Further evidence for nutrient limitation within the Australian sea lion gut, specific to life in an ocean environment, is found in the over-representation of phosphorus metabolism and iron scavenging mechanism genes compared to both Fish A and Human A microbiomes. Iron is the limiting nutrient for many open ocean ecosystems [22] and increased iron uptake ability and phosphorus metabolism potential may allow for marine organisms to survive in ecosystems low in these essential nutrients. Foraging in the nutrient poor open ocean may have influenced the metabolism of the Australian sea lion gut microbiome in such a
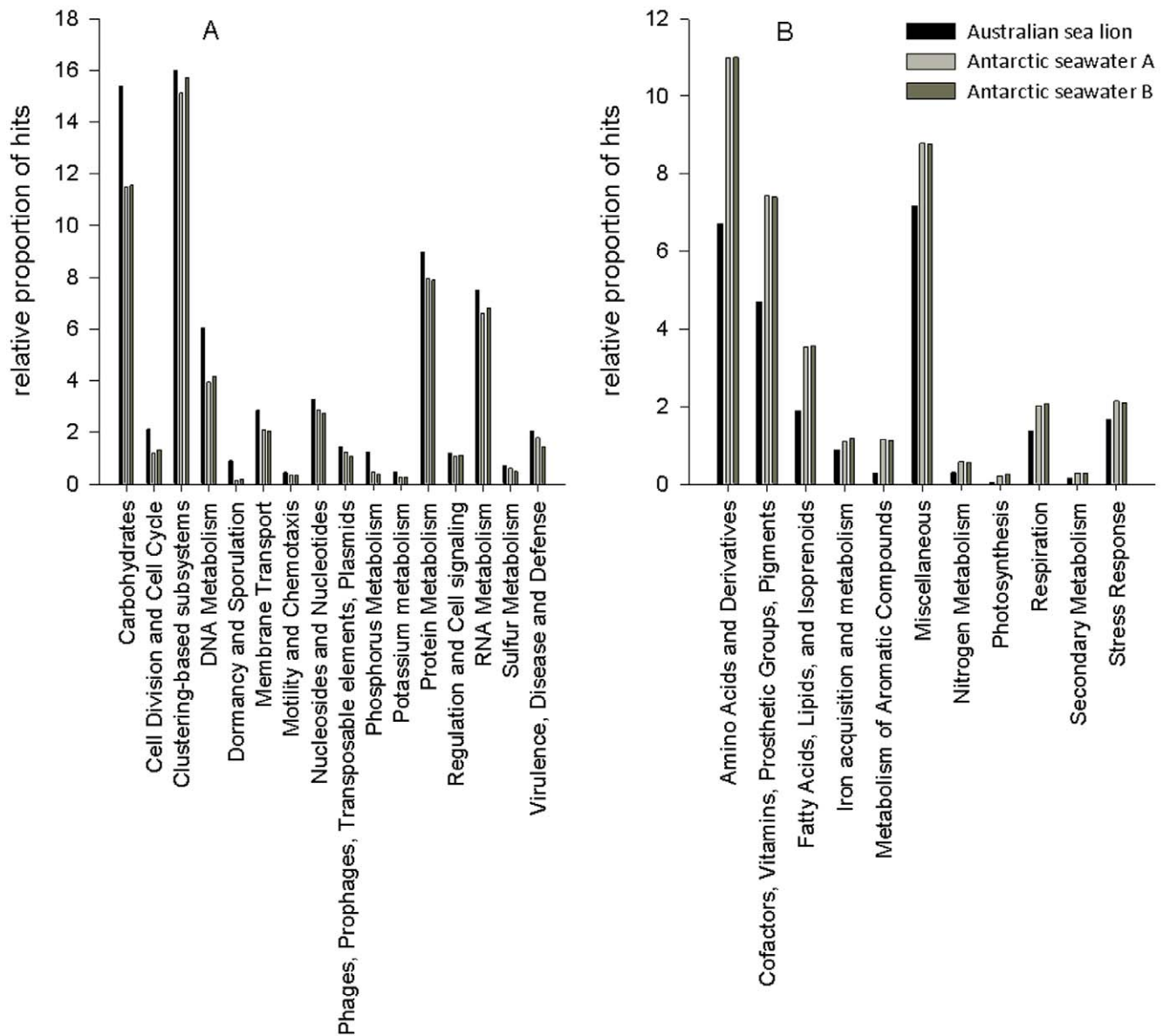
Figure 5. Metabolic Subsystems Over-represented and Under-represented in the Australian Sea Lion Faecal Microbiome compared to two Antarctic Seawater Microbiomes. A: The metabolic subsystems that are over-represented in the Australian sea lion faecal microbiome compared to two Antarctic seawater microbiomes. B: The metabolic subsystems that are under-represented in the Australian sea lion faecal microbiome compared to two Antarctic seawater microbiomes.
doi:10.1371/journal.pone.0036478.g005

way as to ensure maximum uptake and metabolism of the limiting and valuable nutrients necessary for growth and reproduction.

Compared to the Antarctic seawater microbiomes, the Australian sea lion faecal microbiome was over-represented in 16 processes including phosphorus metabolism, potassium metabolism, sulphur metabolism and genes involved in virulence, disease and defence. Similar to comparisons with Human A and Fish A microbiomes, the Australian sea lion faecal microbiome was again over-represented in genes coding for membrane transport, cell division and carbohydrate metabolism (Figure 5A). The Australian sea lion faecal microbiome was under-represented in 11 metabolic processes including iron acquisition and metabolism, nitrogen metabolism, photosynthesis, respiration and metabolism of aromatic compounds when compared to both Antarctic seawater samples (Figure 5B).

Environmental Consequences of Australian Sea Lion Defecations

The enriched number of genes coding for phosphorus metabolism in Australian sea lion faeces compared to Human A, Fish A and Antarctic seawater samples may have important environmental consequences if the sea lion faeces is defecated into surface waters. Bacteria require carbon, phosphorus, nitrogen and micronutrients including iron for growth and are net consumers of these nutrients in energy-poor environments. However, in nutrient-rich environments like the surface of a faecal particle, bacteria can solubilise more Fe, P and N from faecal matter than they require for their own growth (uncoupled solubilisation) [23]. This leads to leaching of these nutrients into the surrounding waters [23] where they can become available for free living

microbes. Therefore, the bacteria in Australian sea lion faeces may limit nutrient sinkage to depth and enhance the persistence of nutrients in the photic zone where they are available to support primary production by phytoplankton [24].

Conclusion

This metagenomic analysis reveals the genetic content and metabolic potential of an Australian sea lion gut microbiome. The phylogeny of the Australian sea lion gut microbiome is characterised by a high *Firmicutes* to *Bacteriodetes* ratio, which indicates a predisposition towards excess body fat in other mammals. The metabolic potential of the Australian sea lion gut microbiome was more similar to human gut microbiomes than cow gut, chicken cecum, fish guts, seawater samples or whale fall microbiomes. Compared to a human gut microbiome, the Australian sea lion gut microbiome had enriched numbers of genes coding for iron scavenging mechanisms and phosphorus metabolism. This finding suggests that Australian sea lion faeces contains bacteria able to assimilate and metabolize nutrients and is an important addition to the developing research showing that marine mammal faeces contribute to ocean nutrient dynamics.

Supporting Information

Figure S1   Statistical Differences in Taxonomic Diversity between Australian Sea Lion and Human A Faecal Microbiomes. Symbols to the right are metabolic subsystems that are over-represented in the Australian sea lion (•) faecal microbiome compared to the Human A faecal microbiome. Symbols to the left are over-represented in the Human A (•) faecal microbiome compared to the Australian sea lion faecal microbiome.
(PDF)

Figure S2   Statistical Differences in Metabolic Potential between the Australian Sea Lion and Human A Faecal Microbiomes. Symbols to the right are metabolic subsystems that are over-represented in the Australian sea lion (•) faecal microbiome compared to the Human A faecal microbiome. Symbols to the left are over-represented in the Human A (•) faecal microbiome compared to the Australian sea lion faecal microbiome.
(PDF)

Figure S3   Statistical Differences in Taxonomic Diversity between Australian Sea Lion and Fish A Faecal Microbiomes. Symbols to the right are metabolic subsystems

that are over-represented in the Australian sea lion (•) faecal microbiome compared to the Fish A faecal microbiome. Symbols to the left are the metabolic subsystems over-represented in the Fish A (•) faecal microbiome compared to the Australian sea lion faecalmicrobiome.
(PDF)

Figure S4   Statistical Differences in Metabolic Potential between the Australian Sea Lion and Fish A Faecal Microbiomes. Symbols to the right are metabolic subsystems that are over-represented in the Australian sea lion (•) faecal microbiome compared to the Fish A faecal microbiome. Symbols to the left are over-represented in the Fish A (•) faecal microbiome compared to the Australian sea lion faecal microbiome.
(PDF)

Figure S5   Statistical Differences in Metabolic Potential between the Australian Sea Lion and Antarctic Seawater A Microbiomes. Symbols to the right are metabolic subsystems that are over-represented in the Australian sea lion (•) faecal microbiome compared to the Antarctic Seawater A microbiome. Symbols to the left are over-represented in the Antarctic Seawater A (•)microbiome compared to the Australian sea lion faecal microbiome.
(PDF)

Figure S6   Figure S6. Statistical Differences in Metabolic Potential between the Australian Sea Lion and Antarctic Seawater B Microbiomes. Symbols to the right are metabolic subsystems that are over-represented in the Australian sea lion (•) faecal microbiome compared to the Antarctic Seawater B microbiome. Symbols to the left are over-represented in the Antarctic Seawater B (•)microbiome compared to the Australian sea lion faecal microbiome.
(PDF)

Acknowledgments

Author Contributions

Conceived and designed the experiments: TJL BR JS JGM TJ. Performed the experiments: TJL BR TJ. Analyzed the data: TJL BR TJ. Contributed reagents/materials/analysis tools: TJL JGM. Wrote the paper: TJL.

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Predation on an Upper Trophic Marine Predator, the Steller Sea Lion: Evaluating High Juvenile Mortality in a Density Dependent Conceptual Framework

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Abstract

The endangered western stock of the Steller sea lion (*Eumetopias jubatus*) – the largest of the eared seals – has declined by 80% from population levels encountered four decades ago. Current overall trends from the Gulf of Alaska to the Aleutian Islands appear neutral with strong regional heterogeneities. A published inferential model has been used to hypothesize a continuous decline in natality and depressed juvenile survival during the height of the decline in the mid-late 1980's, followed by the recent recovery of juvenile survival to pre-decline rates. However, these hypotheses have not been tested by direct means, and causes underlying past and present population trajectories remain unresolved and controversial. We determined post-weaning juvenile survival and causes of mortality using data received post-mortem via satellite from telemetry transmitters implanted into 36 juvenile Steller sea lions from 2005 through 2011. Data show high post-weaning mortality by predation in the eastern Gulf of Alaska region. To evaluate the impact of such high levels of predation, we developed a conceptual framework to integrate density dependent with density independent effects on vital rates and population trajectories. Our data and model do not support the hypothesized recent recovery of juvenile survival rates and reduced natality. Instead, our data demonstrate continued low juvenile survival in the Prince William Sound and Kenai Fjords region of the Gulf of Alaska. Our results on contemporary predation rates combined with the density dependent conceptual framework suggest predation on juvenile sea lions as the largest impediment to recovery of the species in the eastern Gulf of Alaska region. The framework also highlights the necessity for demographic models based on age-structured census data to incorporate the differential impact of predation on multiple vital rates.

Introduction

The endangered, western population segment of the Steller sea lion (*Eumetopias jubatus*) has declined to about 20 percent of peak levels recorded four decades ago, with locally divergent but overall stable trends in the Gulf of Alaska (GOA) and Bering Sea - Aleutian Islands (BSAI) [1]. The formerly less abundant, threatened eastern population (east of 144° West longitude) has increased about 3% *p.a.* during this period from South-east Alaska through California [1,2]. Other upper trophic level mesopredators in the GOA and BSAI, including northern fur seals (*Callorhinus ursinus*), harbor seals (*Phoca vitulina*) and sea otters (*Enhydra lutris*) have also exhibited precipitous declines through portions of their range [3–7].

Several hypotheses describing forcing on these mesopredators in the GOA – BSAI region have been advanced, including the resource-driven junk food [8] and ocean climate [9] hypotheses, and the consumer-driven sequential megafaunal collapse hypothesis. The latter suggests a cascading prey shift in transient killer whales (*Orcinus orca*) triggered by the collapse of their former prey, the great whales, through commercial whaling [3,10].

Resource driven hypotheses primarily postulate changes in abundance, distribution and accessibility, composition or nutritional quality of prey [8,11]. These changes may be natural (i.e. driven by episodic changes in ocean climate) or anthropogenic (i.e. through large scale industrial fishing). Such bottom-up effects are thought to reduce fitness primarily through negative impacts on overall energy budgets of individual animals. When energetic demands associated with homeostasis or growth, foraging and reproduction are not balanced by energy gained through prey consumption, the ability to grow, survive or reproduce is compromised. Consumer driven hypotheses postulate direct causes of individual animal mortality independent of energetic balance [8,11]. Predation, incidental mortality in fishing gear, ship strikes, subsistence harvest and illegal shooting comprise such top-down forces [8,11,12]. Pollutants and diseases may fall into either category. Lethal pathogens and pollutants can directly affect survival, while non-lethal agents can indirectly affect growth and

reproduction through altered energy balance. Pollutants can also directly affect reproduction by depressing fertility [13]. Furthermore, poor health and condition, and unbalanced energy budgets may compromise an individual's ability to evade predation. Effects in both top-down and bottom-up categories may exhibit density dependent and density independent characteristics. However, resource effects commonly exhibit density dependence at high abundance, whereas consumer effects are apt to be density independent at high abundance with possible density effects at low abundance [11].

Observations and physical evidence demonstrate the occurrence of predation on declining mesopredator populations in the GOA and BSIA [14–18], primarily by the transient ecotype of the killer whale [14], but also by salmon sharks (*Lamna ditropis*) and Pacific sleeper sharks (*Somniosus pacificus*) [12]. From a review of data collected through the 1990s the National Research Council concluded that recovery of the western Steller sea lion population was more likely limited by predation than by resource driven effects [11].

However, through indirect evidence interpreted in support of resource and other constraints on Steller sea lion productivity [19–22,1], attention has recently shifted towards anthropogenic and natural bottom-up forcing, possibly expressed as reduced reproductive rates or natality. For the purposes of our study, we define natality as the number of female *and* male pups born divided by the number of females of reproductive age. Perhaps most influential in current discussions of sea lion population trajectories, Holmes and collaborators [21] used a time-varying Leslie population matrix to model vital rates for the central GOA region of the western Steller sea lion. Aerial survey photographs were used to estimate population age structure from animal length class distributions. Model demographic outputs were compared to observed abundances also obtained from aerial photographs. Fecundity schedules (adult females) and survival schedules (separately for juveniles and adults) from the central GOA region during the late 1970's used in the Leslie matrix were scaled by time periods to obtain best fits to observed population trajectories and juvenile fraction age structure metrics. Thus, the model yielded scaling factors by time periods for changes in natality, survivorship and age structure. From the best fitting inferential model the authors derived the following hypotheses: (1) natality steadily declined from 67% in the 1970's to 43% by 2006; (2) juvenile survival was depressed during the height of the decline in the mid 1980's; (3) juvenile survival recovered to pre-decline rates by 2006. Though only central GOA demographics were modeled, the authors proposed applicability of their hypotheses across the entire GOA and AI region.

However, all current hypotheses on forcing remain untested by direct measures, and factors driving past and present population trajectories are undetermined [1,10,22–24], largely due to immense logistical constraints on working with a large and effectively cryptic marine mammal, the Steller sea lion. Despite this absence of empirical testing, the vital rate changes proposed by Holmes et al. have been broadly embraced as the conceptual centerpiece of policy and management,, resulting in changes to research priorities and the recovery plan. In 2010, Maniscalco et al. [25] provided a direct assessment of Steller sea lion natality in the eastern GOA based on a longitudinal study of n = 151 individual females observed at the Chiswell Island rookery, and estimated natality at 69% (+/−2.5% S.E.). With their empirical evidence contradicting the hypothesized decline in natality at least for the eastern and likely central GOA region (Chiswell Island is located near the boundary between the eastern and central GOA regions), the authors concluded that 'alternative hypotheses must

be more seriously considered' [25]. It is possible that the primary components of the past population changes may never be understood, but advances in tracking technology now provide an opportunity to directly measure aspects of sea lion biology that were previously 'empirically intractable' [17], specifically causes and rates of mortality.

To directly measure mortality and predation in the western Steller sea lion, we deployed specialized telemetry transmitters [26] in n = 36 juvenile sea lions from 2005 through 2011 in the Kenai Fjords and Prince William Sound region of the GOA. The abdominally implanted [27] archival Life History Transmitters (LHX tags) record data throughout the host's life. LHX tags primarily rely on sensor data from temperature, light, and dielectric properties of surrounding medium to determine host state [26]. After death, positively buoyant tags liberated from decomposing or dismembered carcasses, or passed through the digestive tract of predators will come to float on the ocean or rest ashore, and will then transmit stored data to orbiting satellites [26–28]. Transmitted data on light levels, surrounding medium, temperature profiles recorded across mortality events and time to transmission allow distinction of predation (rapid temperature drop, immediate sensing of air and light, immediate transmissions) from non-traumatic deaths (gradual temperature decline while surrounded by tissue, delayed sensing of light and air and onset of transmissions) [28]. To increase data recovery likelihood and estimate event detection probability, 34 of 36 animals received two implants.

We place our measures of post-weaning mortality and predation into the context of forcing by means of a qualitative conceptual framework. The framework integrates age structured, density dependent effects with density independent effects on survival, reproduction and population trajectories. From the combination of previously unavailable empirical data and qualitative conceptual framework we propose an alternative hypothesis to the postulated depressed natality for present day forcing of the Steller sea lion population in the eastern GOA.

Materials and Methods

Ethics Statement

This study was carried out in strict compliance with all applicable Animal Care and Use Guidelines under the U.S. Animal Welfare Act and was approved as required under the U.S. Marine Mammal Protection Act and the U.S. Endangered Species Act by the U.S. National Marine Fisheries Service (Permit numbers 1034–1685, 1034–1887, 881–1890, 881–1668, 14335, 14336) and by the Institutional Animal Care and Use Committees of the Alaska Sea Life Center (02-015, 03-007, 05-002, 06-001, 08-005, R10-09-04), and Texas A&M University (2003-181, 2005-170, 2006-37). All surgeries were performed under aseptic conditions and under full inhalant gas anesthesia, and all efforts were made to minimize pain and suffering.

Animals, procedures and controls

Thirty six juvenile Steller sea lions (*Eumetopias jubatus*) were captured in Prince William Sound (PWS), Alaska from 2005 to 2011. Capture, transport to, temporary holding and husbandry at a quarantined facility at the Alaska SeaLife Center (Seward, Alaska) were performed as previously described [29,30]. Intraperitoneal implantation of LHX tags (technical details in [26]) occurred under gas anesthesia using standard aseptic surgical procedures as previously described [27]. The first two animals received single LHX tag implants (2005), all subsequent animals received dual transmitter implants to facilitate estimation of data

recovery probability. Post-operative clinical, physiological and behavioral monitoring lasted 1–6 weeks [27,30–32]. All animals were released into Resurrection Bay in the Kenai Fjords area (2005 n = 2, 2006 n = 4, 2007 n = 5, 2008 n = 10, 2009 n = 6, 2010 n = 5, 2011 n = 4). All animals but one were hot-iron branded prior to release as per Mellish et al. [30]. All animals (male n = 28, female n = 8) were greater than 12 months of age and weaned at the time of capture. At the time of release, animals ranged from 13 to 22 months with the exception of one individual (25 months). Extensive post-surgical health assessments showed a typical mild to moderate immune and stress response to the procedure [30,31]. All clinical health parameters monitored returned to baseline values within six weeks of surgery [30,31], from which we derived a 45 day post-implant 'confirmation of survival' criterion for inclusion of individual animals in this study.

To confirm short-term survival, and to compare foraging and ranging behavior of LHX tag recipients to 23 non-implanted, temporarily captive control animals, 35 of the 36 LHX sea lions were monitored after release via externally attached, satellite-linked data transmitters [30,33]. Minimum confirmed post-surgery survival was derived from external transmitter data, opportunistic re-sights of individual brands or LHX tag data over a range 47 to 2,072 days (mean 534+/−87.5 s.e.m., n = 36). All 36 study animals had survival confirmed beyond the 45 day criterion and are included in the results reported here. As previously reported, post release diving and ranging behavior did not differ between LHX tag recipients and non-implanted control animals [30], or between controls and free-ranging juveniles [33].

To evaluate potential long-term impacts of tags and surgeries on survival, we compared LHX-based survival rate estimates (see below) to rates derived from hot-iron brands applied to n = 255 juvenile sea lions in PWS by the National Marine Fisheries Service from 2001–2005, with re-sight surveys conducted from 2002–2008 (these data were evaluated using a Cormack-Jolly-Seber open population model in the program MARK [34], and were provided as sex specific annual survival rates and uncertainties by L. Fritz, pers. comm.). Sex-specific cumulative survival rates were obtained as products of sequential annual rates and weighted to reflect a similar sex ratio as the LHX study. Uncertainties were similarly obtained as weighted products of age and sex specific annual confidence limits.

Estimation of mortality detection probability and survival rates

To estimate survival rates from LHX tag data, detected mortality events need to be corrected for events not detected due to failure of devices to successfully uplink to the ARGOS system aboard NOAA satellites. Uplink failures are the combination of technical tag failures and transmissions from a functional tag not reaching any satellite due to tag exposure constraints [26,28]. Tag uplink failure probability was estimated from the ratio of single to dual LHX tag data returns from dual tag deployments (n = 34 live animal, n = 9 carcass test). The tag uplink failure probability P_{fail} can be calculated as $P_{fail} = C_{single}/(C_{single} + 2C_{dual})$ where C_{single} is the count of single returns, and C_{dual} is the count of dual returns. A correction factor F can then be derived as $F = 1/(1-P_{fail}^2)$ and the corrected number of mortality events E_{corr} calculated as $E_{corr} = F (C_{single} + C_{dual})$. From P_{fail} the event detection probability P_{detect} can in turn be derived as $P_{detect} = 1-P_{fail}^2 = 1/F$ [28]. Ranges containing 95% of likely variance for the estimate of P_{fail} were derived from the Cumulative Distribution Function of a Monte Carlo simulation (>2,500 iterations) of randomly assigned individual tag failures for $0<P_{fail-simulated}<1$ yielding P_{fail} not exceeding the observed value without increasing

E_{corr} integer counts. The 95% confidence range for P_{fail} in turn yields confidence intervals for F and E_{corr}.

We calculated daily mortality rates from age-class specific subsets of cumulative exposure days (d_{exp}) and corrected mortality counts as $DMR = E_{corr}/d_{exp}$. We then calculated daily survival rates $DSR = 1 - DMR$, and rates for periods longer than one day were obtained by raising DSR to the power of period duration in days (i.e. annual survival rates are $DSR^{365.25}$) [35,36]. 95% confidence limits can be calculated from variance and standard error as per Johnson [36]. However, the Johnson method does not include the effects of P_{fail} on the estimation of E_{corr}. To include the effects of uplink failures on estimation of survival rate confidence limits, we used the upper confidence limit for E_{corr} (and correspondingly reduced d_{exp}) to re-compute the lower survival rate confidence limit as per Johnson (upper survival rate limits remain unchanged). Though a total of 29,581 exposure days were logged from 36 animals (ages of 13–90 months), complete year classes for >60 months were covered by only 5 animals (1,825 days for each year class) and inclusion of ages >60 months would substantially increase uncertainties. Therefore, only data from 24,072 cumulative exposure days over the ages of 13–60 months are used here (13–24 months: 35 animals×5,757 d_{exp}; 25–36 months: 25 animals×7,763 d_{exp}; 37–48 months: 18 animals×5,979 d_{exp}; 49–60 months: 15 animals×4,573 d_{exp}).

Determination of causes of mortality

Causes of mortalities were inferred from temperature data recorded across mortality events, concurrent changes in surrounding medium (organic tissue, saltwater or air), time to sensing of light and onset of transmissions, and ancillary data as previously reported [28]. Gradual cooling and delayed extrusion are indicative of non-traumatic deaths (i.e. disease or starvation), or of entanglement, drowning or shooting. An *algor mortis* (body cooling) computational model parameterized for sea lions and validated through carcass testing allows the distinction of cooling masses if well outside of model uncertainties [28]. Precipitous drops to ambient temperature with immediate sensing of light and onset of transmissions are indicative of acute death by massive trauma associated with dismemberment by predators leading to the immediate release of tags [28]. Ship strikes, entanglement, drowning and shooting have been reported for the BSAI region [12]. Ship strikes on marine mammals are usually described as massive blunt force trauma but like drowning and shooting are unlikely to result in an immediate extrusion of both tags [28]. Thus, all acute and non-traumatic events *other than* predation should lead to a gradual transition to ambient temperatures as the entire carcass cools, with substantially delayed tag extrusion and onset of transmissions, unless the tags are liberated by immediate dissection.

To provide uncertainties for the estimated proportion of mortalities by predation PP, we conducted a Monte Carlo simulation of n mortalities (where n is the number of events detected for which cause of mortality could be determined) for simulated values of $0<PP<1$ set in increments of 0.1 (10,000 iterations). The lower confidence limit was then calculated as 95% of the Cumulative Distribution Function of the actual number of predation events out of the number of detected mortalities.

A simplified Leslie matrix to estimate age structured, cross sectional predation and minimum natality

To estimate cross-sectional, age structured consumption of sea lions by predators, we derived an updated contemporary survival schedule for a birth-pulse Leslie population matrix, separately for each sex (Table S1). The matrix uses a schedule of annual survival

rates for ages from 1 month to 31 years, but excludes a fecundity schedule, since no accurate recent estimates exist for age specific adult female fecundity (see introduction). In a standard Leslie matrix, fecundity is used to estimate pup production and overall natality. Sequential matrix years are then seeded with pup production from preceding years to generate outputs for time-varying simulations of population trajectories [37,21]. By excluding fecundity, the simplified matrix cannot be used to model time-variant population trajectories. However, even without fecundity the simplified matrix can be used to estimate *minimum natality* for conditions of stable or increasing population trajectories. The *minimum natality* yields an *equilibrium survivorship schedule* with a theoretical Eigenvalue of 1 for a corresponding time-variant matrix. Assuming that primiparity occurs at the average age of 5.3–5.6 years (after first ovulation at 4.3–4.6 yrs; [38,25]), and that females reproduce through the age of 21 years but not beyond, *minimum natality* is estimated as the pup seed count divided by the number of females between the ages of 5 and 21 (inclusive). This assumed reproductive age span for adult females is consistent with the fecundity schedule used by Holmes et al. [21]. This simplistic measure does not require assumptions about age specific fecundity (i.e. any decline in natality with parity for old females), and the estimate is therefore independent from the age structure of the adult population, but is only applicable to stable or increasing populations.

We modified the best fit survival schedule from Appendix C of Holmes et al. [21] as adjusted by the authors using their best fit scaling factors for the 1998–2006 period. This schedule is denoted HFYS-06 in Table S1. The original, un-scaled survival schedule for pre-decline conditions used by Holmes et al. is also listed as HFYS-Pre. We modified the HFYS-06 schedule with survival rates for ages 13–60 months replaced by LHX-based estimates. We also replaced survival rates for ages 1–12 months with values averaged from Pendleton et al. [39] and Maniscalco et al. [40,41]. This value used for young-of-the-year matches brand-resight based estimates by the National Marine Fisheries Service (L. Fritz, pers. comm.). Thus, survival rates in HFYS-06 for ages 1–60 months (the youngest 5 years) were replaced with more recent and location-specific estimates. This replacement however resulted in an improbably high *minimum natality* estimate of 0.92 compared to 0.6 for the unmodified HFYS-06 matrix (Table S1). To correct for this shift, we altered the scaling factor for adult survival from 1.07 (as used by Holmes et al. [21]) to 1.13 to produce a minimum natality estimate of 0.69, the value reported by Maniscalco et al. for the eastern Gulf of Alaska [25]. This resulted in the LHX-eGOA schedule listed in Table S1. The LHX-eGOA schedule uses identical values for males and females for ages 1–60 months, and assumes a 1:1 sex ratio at birth. For males >60 months a survival schedule was generated as a progressively decreasing proportion of female rates to match sex-specific age frequency distributions to values reported for n = 235 males and n = 282 females collected from 1976–1989 by Calkins and Pitcher [42]. The sex specific schedules resulted in 95% of females in the population comprised within ages 1–19 years, and 95% of males within ages 1–14 years.

We added age-class specific estimates for the proportion of mortality by predation *PP* (see above), and from that in turn derived two mortality schedules separately for each sex, one for predation and one for all other sources of mortality. We used our LHX-based *PP* estimate for juveniles (ages 13–60 months). For young-of-the-year (ages 1–12 months) we used 30% of the *PP* value for juveniles to obtain predation rates comparable to values reported for the eastern GOA in literature [40]. For age classes >60 months we reduced the juvenile *PP* by 50% *p.a.* to account

for a hypothesized reduction in vulnerability to predation with increasing age, size and experience (i.e. [17,43], and see discussion). This resulted in adult predation accounting for only 4% of all predation events in females and 5% in males. As in a standard Leslie matrix, population vectors with absolute counts of animals in each age class can be generated from a birth-pulse (seed count of pups) and the survival schedule. Similarly, predation and non-predation vectors can be generated from the mortality schedules listed in Table S1.

A conceptual, qualitative population model to combine density-dependent with density-independent effects

To evaluate the potential impact of the observed levels of post-weaning mortality by predation, we constructed a qualitative conceptual population model to integrate age structured, density dependent consumption of sea lions by predators with density independent mortality by other causes. We used the contemporary LHX-eGOA schedules (Table S1) to calculate vectors for population numbers, consumption by predators and non-predation mortalities for specific abundance levels (vector sums) from 0% to 100%. Population vector sums were adjusted through selection of appropriate pup seed counts (birth pulses) with 100% abundance set to the peak historic level of approximately 180,000 animals, and the recent population estimates of approximately 36,000 animals used for the 20% contemporary abundance (western U.S. stock [1,44]). Non-predation mortality was assumed not to vary with density and the m_{npi} schedule and corresponding mortality vectors therefore remained identical for all abundance levels. Numerical consumption by predators was adjusted as a function of sea lion abundance according to three different, age-structured response types (*Flat, Linear, Sigmoid*), yielding adjusted predation vectors. From the combined predation and non-predation vectors, an updated survivorship schedule was computed. Since this density-dependent model is not a time-variant Leslie matrix and has no fecundity schedule, a population trajectory cannot be calculated. However, from the sum of the female population vector for the ages of reproductive maturity (5–21 years, see above) multiplied by an assumed birth rate pup production can be estimated. We calculated the *potential trajectory* for a given abundance as the difference of the birth pulse seed count minus the actual pup production for a set birth rate.

A number of additional metrics were computed for comparative purposes: the total number of animals consumed for a given abundance (the numerical response) was calculated as the sum of the predation vectors for both sexes. An estimation of total sea lion mass consumed by predators was obtained by multiplying the predation vectors with an age-structured mass schedule separately for each sex. We used the mass schedules from Table 3 of Winship et al. [45], and used a mean mass of pregnant and non-pregnant females of reproductive age weighted by the proportion of females pregnant (the set birth rate). The juvenile fraction J/T was calculated as the population vector sums for ages 2–5 years, divided by the population vector sums for ages 2–31 years, for both sexes. Female recruitment was calculated as the percentage of the female seed count surviving to the end of year 4.

Results and Discussion

Detected mortalities and survival rates

Data from twelve detected mortality events were received from November 2005 through November 2011 during 24,072 cumulative exposure days. Seven events occurred within ages 13–24 months, four events within 25–36 months, and one event at age 49 months. From the ratio of dual LHX tag data returns (n = 9 from

12 detected mortalities plus n = 8 from 9 carcass simulations) to single returns (n = 3 live plus n = 1 carcass), we estimated tag uplink failure at $P_{fail} < 0.105$ and mortality event detection probability at $P_{detect} > 0.989$ (95% c.i. 0.0.92–1.0). Within these returns, the joint probability distribution of live animal and carcass returns gave an odds ratio of 2.7 (odds ratio test [46]), suggesting no differences in detection probabilities between live animal and carcass deployments (Fisher Exact Probability $P_{(2,1)} = 0.6$ [47]). From the correction factor $F = 1.0112$ we derived the corrected mortality count of $E_{corr} = 12.13$ (95% c.i. 12–13). However, since animals cannot die by fractional numbers, we used the integer portion of $E_{corr} = 12$ for subsequent calculations.

We estimated the cumulative survival for ages 13–36 months at 0.531 (95% c.i. 0.40–0.63, Table 1). This combined rate for both sexes is slightly lower than the control estimate of 0.534 based on brand re-sights but with overlapping confidence limits of (95% c.i. 0.42–0.64). Our annual estimates are on either side of controls (in parentheses) for subsequent year classes: 13–24 months: 0.641 (0.690), 25–36 months: 0.829 (0.775), 37–48 months: 1.0 (0.884); 49–60 months: 0.923 (0.875). Our estimate for the cumulative survival for ages 13–60 months is 0.491 (Table 1). Since LHX tags deliver event data with a resolution <1d, survival rate estimation only requires inferences on dates of undetected events, less than 3% of events or 1.5% of animals in our study. Brand re-sight based survival estimates require inferences on dates for all apparent mortalities, more than 50% in the control study by the National Marine Fisheries Service. For annual rates or re-sight efforts unknown dates are inferred to +/−182.5d. This difference explains the comparable uncertainties for these two distinct methods with sample sizes that differ by almost one order of magnitude. Furthermore, LHX tags provide information not only on dates, but on locations and causes of mortality of individual animals. Within the limits of the uncertainties for both techniques, the survival rate comparison does not suggest any impact of LHX tags, surgeries or temporary captivity on survival for ages 13–60 months.

Mortalities occurred in two of eight monitored females and ten of 28 monitored males. The odds ratio was 1.43, suggesting no differences in mortality probabilities between sexes within the data set (Fisher Exact Probability $P_{(2,1)} = 1.0$). Mortalities occurred 1 each in August, September, October, November and March; 2 each in January and May; and 3 in February.

Event location accuracy from four events with pre-mortality locations from external tracking devices was estimated at 10.4 km (+/−3.1 s.e.m.) [28]. Eleven of the twelve detected mortalities occurred within the previously described geographic range covered by implanted and non-implanted juvenile Steller sea lions following release from temporary captivity [30,33] (Fig. 1). One
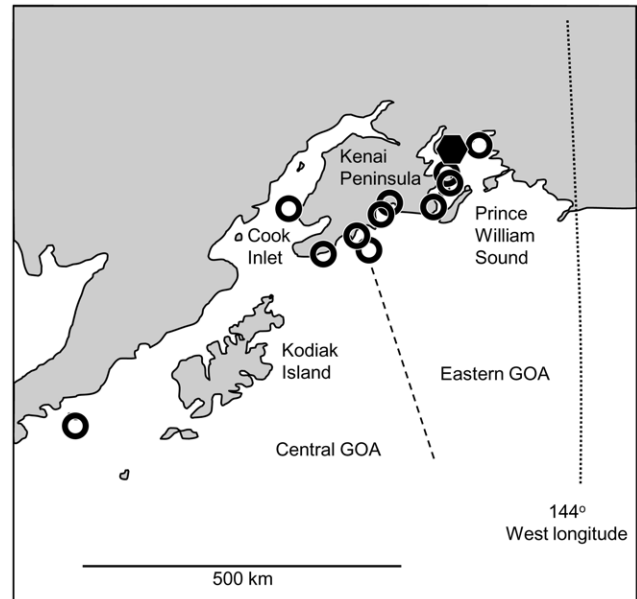

Figure 1. Locations of juvenile Steller sea lion mortalities detected in the Gulf of Alaska. Eleven predation events indicated by open circles occurred in the eastern and central Gulf of Alaska (GOA). One mortality of undetermined cause indicated by the solid hexagon occurred in Prince William Sound. The endangered Western Distinct Population Segment is located to the west of 144° longitude.
doi:10.1371/journal.pone.0030173.g001

event occurred outside of this area, to the west of Kodiak Island, though this is still within known ranges of juvenile sea lions [48]. No mortalities occurred near rookeries, and four of the twelve events occurred near juvenile-dominated haulouts.

Causes of mortality

One of twelve detected events (in age class 25–36 months) provided no data other than confirmation of mortality and event date, due to a technical failure in the single LHX tag of the implanted pair that successfully uplinked. Eleven events provided data including death time stamps, time from death (determined as per [26,28]) to onset of transmissions, and complete 48 hour temperature profiles across the mortality events. Ten events exhibited precipitous temperature drops with immediate tag extrusion and onset of transmissions, indicative of predation. One event exhibited gradual cooling with *algor mortis* model outputs corresponding to 14% of predicted mass, suggesting partial dismemberment, most likely due to predation [28]. Thus, all eleven detected events that yielded data were classified as predation events. The combined probability distribution of single to dual tag returns for these eleven predation events and the nine carcass tests that constitute simulated non-traumatic deaths (2 in 9 for predation and 1 in 8 for non-traumatic deaths) gave an odds ratio of 0.56 suggesting no differences in detection probabilities between predation versus non-traumatic events (Fisher Exact Probability $P_{(2,1)} = 1.0$). The finding of a minimum of eleven predation events in twelve detected mortalities yields an estimated proportion of juvenile sea lion (ages of 13–60 months) mortalities in the PWS-KF region due to predation of $PP> = 0.917$ (95% c.i. 0.78–1.0). However, we used the center of the 95% confidence interval of $PP = 0.89$ for all subsequent model calculations. Given that most observations of Steller sea lions typically occur during the breeding season (June–September) with focus on rookeries [16,40,49]), the timing and location of our detected mortality

Table 1. Cumulative survival for juvenile Steller sea lions for ages 13 through 60 months estimated by different methods.

Model and Period	13–36 months	13–48 months	13–60 months
LHX-eGOA 2005–2010	0.531 (0.40–0.63)	0.531 (0.43–0.60)	0.491 (0.40–0.54)
HFYS-06 1997–2006	0.72 (0.70–0.77)	0.67 (0.65–0.72)	0.61 (0.59–0.66)
HFYS 1983–87	0.42 (0.40–0.47)	0.39 (0.37–0.44)	0.36 (0.33–0.40)
HFYS-Pre 1976–82	0.75	0.70	0.64

Model LHX-eGOA is based on LHX transmitter data returns. Models HFYS are from a published inferential model [21] for three distinct time periods. Numbers in parentheses are 95% confidence intervals where available.
doi:10.1371/journal.pone.0030173.t001

events suggest that previously reported predation rates may be greatly underestimated.

Density-dependent predation models

Predator-prey theory defines three primary types of density dependent prey consumption rates for a given predator density, the functional response [50]. Corresponding numerical responses characterize absolute prey consumption numbers. The Lotka-Volterra functional response (Type I) is primarily applicable to sedentary predators (i.e. web spiders). Type II (Hyperbolic) and Type III (Sigmoid) are both theoretically applicable to mobile marine apex predators and their prey, although their functional responses have not been empirically characterized. Other, less common functional response types exhibit distinct predator-prey dynamics at high densities [51] improbable for marine apex predators. Types II and III are comparable at high densities, but exhibit distinct predator-prey relationships at medium and low prey densities [50–52]. The Hyperbolic response (Type II) is applicable to specialist predators focusing on a single prey species and should result in accelerating prey declines at lower densities which may lead to extinction [52]. The Sigmoid functional response (Type III) is applicable to non-specialist predators that can shift to alternate prey at low prey densities. The Type III functional response results in diminishing prey removal rates at very low densities as predators increasingly switch to alternate prey, in turn creating a prey 'refuge' with increasing survival rates. By comparison, predator-prey systems comprised of pelagic mesopredator fishes and their prey have been studied, and dynamics likely differ from those of marine homeotherms and their apex predators. The dynamically linked populations with density feedbacks of specialized pelagic fishes and their prey are more commonly characterized through a combination of aggregative and numerical responses [51,53,54].

We considered three distinct types of numerical responses between Steller sea lions and their predators. A *Flat* response was generated by not altering the overall and age-class specific prey consumption amounts for abundance levels above 20% (Fig. 2A). The *Flat* numerical response could occur for any type of functional response (Type I, II or III) under the assumption that resource needs of the predator population(s) are fully met at the 20% prey abundance level and saturated above that. A *Linear* response was generated by linearly increasing age-class specific prey consumption amounts from estimates at 20% abundance levels to estimates for the 100% abundance level (Fig. 2B). Consumption estimates for the 20% level were derived as described under methods from the LHX-eGOA schedule. Estimates for the 100% level were derived by setting age-class specific consumption amounts such that the resulting cumulative survival rates for ages 1–5 and 6–10 years matched the HFYS-Pre survivorship schedule (Table S1). The *Linear* numerical response corresponds to a Lotka-Volterra (Type I) functional response rarely seen in non-sedentary predators, but that could occur for highly opportunistic, non-aggregating pelagic predators that pursue a very large variety of prey species, such as sharks. A *Sigmoid* numerical response was generated as a modification of the *Linear* response, by increasing the consumption of juveniles and pups in relation to adults for abundances between 20% and 100% in order to maintain a flat combined consumption of mass for high abundances (Fig. 2C). The *Sigmoid* numerical response corresponds to a sigmoid functional response (Type III) that should be applicable to non-specialist predators such as transient killer whales and possibly sharks. The largely stable mass consumption from 100% down to 60% abundance in our model is concomitant with an increasing consumption of juveniles to compensate for a declining consump-
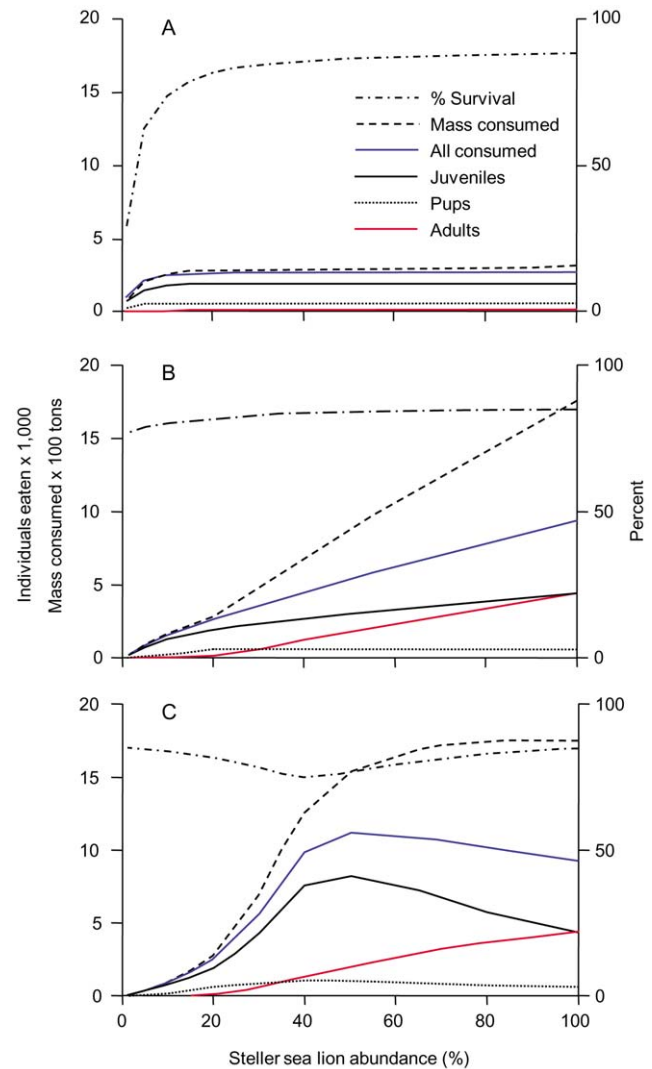

Figure 2. Survival and consumption by predators modeled as a function of Steller sea lion abundance. The percentage of all sea lions surviving to the end of a year, as well as the total mass and numbers of individuals consumed by predators are shown as a function of sea lion abundance, for three distinct numerical response types (see methods and discussion). (A) The *Flat* numerical response. (B) The *Linear* numerical response. (C) The *Sigmoid* numerical response. Numbers of individuals consumed are separately shown for pups (year 1), juveniles (years 2–4) and adults (years 5–31). For the western population, 100% abundance corresponds to 180,000 individuals.
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tion of adults. This differential response by age classes (Fig. 2C) should be driven by the greater vulnerability to predation of younger age classes balanced against the reduced profitability in the form of lower individual mass and energy content (see [17,43]). From an energy content analysis of Steller sea lions, Williams et al. [17] estimated caloric requirements of adult killer whales at 2–3 Steller sea lion pups per day versus one adult female sea lion every 2–3 days. No data exist on the energetic cost or risk of killing and consuming an adult Steller sea lion versus a pup or juvenile. However, the notion of age-structured predation pressure is conceptually supported through a risk-benefit model specifically developed for juvenile Steller sea lions, killer whales and Pacific sleeper sharks by Frid et al. [43]. This model explained seasonal differences in telemetered dive behavior of juvenile Steller sea lions

in PWS through a combination of resource distribution, and higher predation pressure on juveniles than older sea lions. Empirical support for age structured predation pressure on pinnipeds in general is available for Hawaiian monk seals (*Monachus schauinslandi*). From an analysis of 315 shark-inflicted injuries recorded during an 11 year period Bertilsson-Friedman [55] concluded that sharks injured more pups and juveniles than subadults and adults. LHX tag data provides direct evidence of elevated predation risk for younger juveniles at current abundance levels. Since all detected events with sufficient data were classified as predation, the predation probability is the inverse of annual survival rates, or 35.9% for ages 13–24 months, 13.2 to 17.1% for ages 25–36 months depending on actual cause for the single unknown fate event, zero for 37–48 months and 7.7% for ages 49–60 months.

As the density of more profitable adults decreases, juveniles may become increasingly viable alternate prey in a form of intraspecific diet shift. While most marine mesopredators and their prey are distributed in the three-dimensional pelagic zone, the distribution of pinnipeds is constrained in space and time through reproductive activities tied to solid substrate ashore or on ice. This constraint is not uniform across sex and age. Young Steller sea lions are suckled by females at shore-based rookeries and haul-outs, and are typically weaned by the age of one year, with some documented cases of maternal care extended through the ages of 2 or 3 years [38,56]. This results in juvenile age classes (ages 1–36 months) and adult females with increased spatio-temporal constraints in the form of predictable presence near specific shore locations. Within our model, the notion of density dependence of vulnerability and/or spatio-temporal accessibility of juveniles is supported by a comparison of consumption rate estimates between 20% and 100% abundance (Fig. 2C). This comparison is based on the LHX-eGOA (20%) and HFYS-Pre (100%) schedules under the central assumption in our model that non-predation mortality is density independent. At 100% abundance, pups comprise 7% of predation events, juveniles 46% and adults 47%, whereas at 20% abundance, pups comprise 23%, juveniles 72% and adults 5%. This changeover strongly suggests an age structured density dependence in predation rates.

The likely numerical response

The Sigmoid numerical response emerges as the most likely scenario for Steller sea lions and their predators for the following reasons: **(1)** Presently, the western population overall is stable at about 20% of peak abundance [1,44]. The *Sigmoid* model is the only scenario to result in stable trajectories at 20% following a decline (as indicated by the negative slope of the pup difference curve at 20% abundance in Fig. 3). The *Flat* and *Linear* models both exhibit continuing declines. **(2)** Contemporary juvenile survival has only slightly recovered from lowest levels around 40–50% abundance, but is still below peak abundance levels (Table 1). This is the pattern seen in the *Sigmoid* model (Fig. 4C). Both *Flat* and *Linear* models show accelerating declines in juvenile survival with decreasing abundance (Fig. 4A,B). **(3)** The juvenile fraction metric (J/T in Fig. 4) is perhaps the most interesting comparative criterion. Holmes and York [57] provided a retrospective analysis of the juvenile fraction based on length measurements conducted on aerial survey photographs of rookeries and haulouts, for the central GOA. Though their actual ratios based on hauled out animals may differ from our comprehensive estimates for all animals, they reported an increase in the J/T ratio from early- to peak decline, followed by a decrease. This pattern is seen in the *Sigmoid* model, which exhibits a peak in the J/T ratio between 40 and 50% abundance,
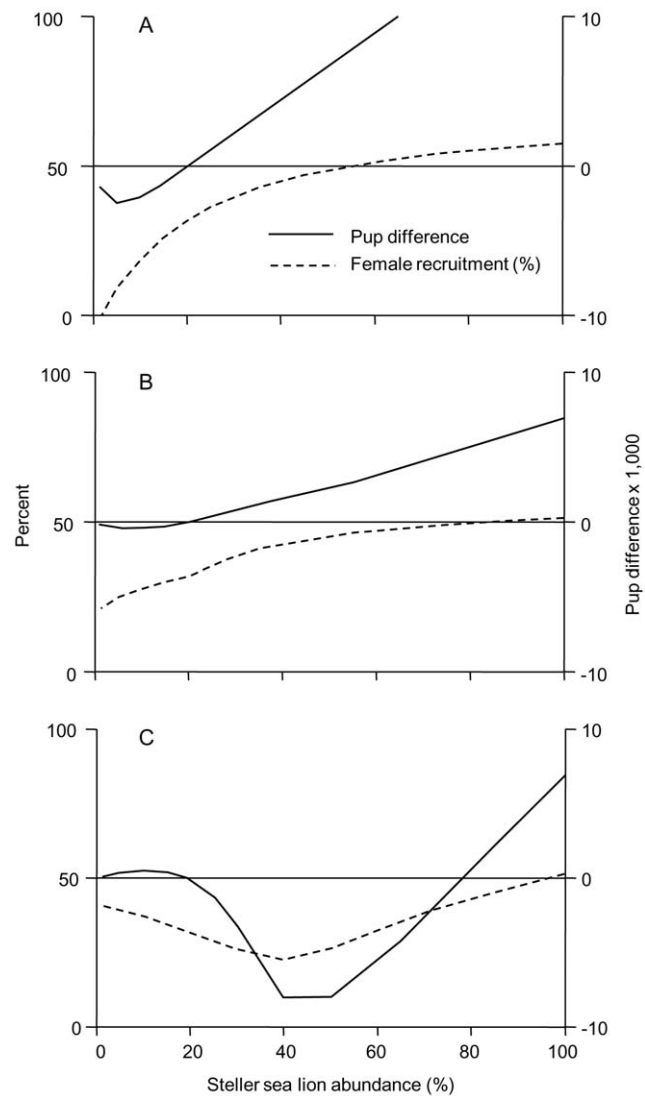
Figure 3. Potential Steller sea lion population trajectory and female recruitment modeled as a function of abundance. The potential population trajectory is calculated as the difference between the birth pulse pup seed count (the theoretical requirement for a stable population) and the actual number of births for a given natality and survivorship schedule - the Pup difference - see text. Female recruitment is the percentage of female pups surviving to the end of year 4. 100% abundance corresponds to 180,000 individuals. (A) The *Flat* numerical response. (B) The *Linear* numerical response. (C) The *Sigmoid* numerical response.
doi:10.1371/journal.pone.0030173.g003

concurrent with the lowest juvenile survival rates and the greatest pup deficit. The *Flat* and *Linear* models conversely show a highly improbable continuing and progressively steeper increase in the J/T metric (*Flat*) or minimal changes down to 40% followed by a very slight drop to a minimum near 20% abundance (*Linear*). Thus, of the three response types considered, the age-structured Sigmoid type is the one most consistent with all available contemporary and retrospective demographic data.

At the contemporary 20% abundance level, model outputs suggest an annual consumption of 2,676 western Steller sea lions or 288 metric tons. This increases to near 11,300 sea lions eaten at 50% abundance or 1,759 tons consumed annually at peak abundance (Fig. 2C). A simple estimate suggests that these
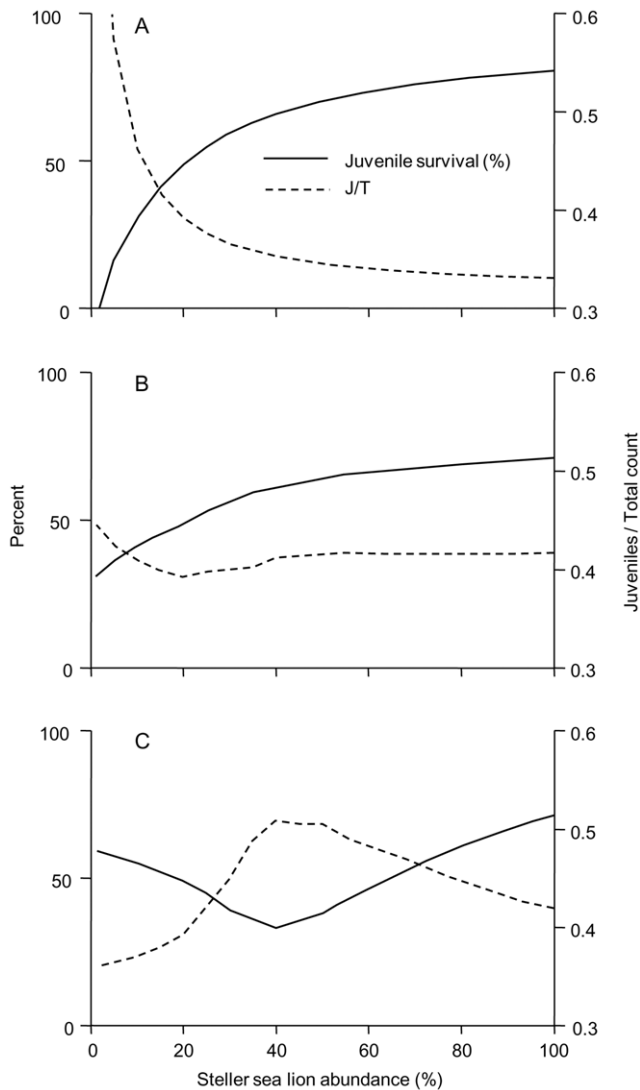
Figure 4. Juvenile survival and the juvenile fraction modeled as a function of Steller sea lion abundance. Juvenile survivorship is shown as the percentage of all juveniles ages 2–4 years that survive to the end of a year. The J/T metric is the count of all juveniles ages 2–4 years divided by the count of all ages 2–31 years (excluding only pups). (A) The *Flat* numerical response. (B) The *Linear* numerical response. (C) The *Sigmoid* numerical response.
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numbers are plausible: combining the current population estimate of 345 transient, mammal eating killer whales for the GOA/BSAI region [58] with the lower of two published theoretical sea lion consumption rates (exclusive of other prey) from caloric requirement estimates [17,40] yields a potential consumption of over 100,000 juvenile Steller sea lions per year. Thus, only about 10% of transient killer whale diet would have to comprise Steller sea lions to yield the modeled effects. The steep increase from 1,938 juveniles consumed annually at 20% abundance to a peak of 8,240 juveniles eaten at 50% abundance shown in Figure 2C may appear as improbably high. However, this increase corresponds to a maximum *PP* of 0.92 at 40% abundance – well within the confidence limits of our contemporary estimate, and a minimum annual survival rate for the most vulnerable age class of 13–24 months of 49%. The latter is within the confidence limits of our

contemporary estimate, and still above the estimated juvenile survival for the height of the decline as per York [37].

Implications of a Type III numerical and functional response

Our framework suggests female recruitment as a key mechanism by which predation may drive the overall reproductive output and the potential trajectory function. From a positive potential trajectory at peak abundance down to the greatest pup deficit at 40–50% abundance, the percentage of adult females directly consumed by predators actually drops from 3.6% to 3.5%, but female recruitment concurrently drops from 51% to only 23% (Fig. 3C). Even without any changes in natality high predation on juveniles could effectively cut the reproductive potential of the population in half. Even if actual natality were to increase above the contemporary regional estimate of 0.69 [25] to 1.0, this would merely shift the equilibrium density from 20% to about 32% abundance. At higher abundance, the potential trajectory function would remain negative. Only significantly reduced consumption of juveniles at intermediate densities would lead to a positive potential trajectory at all abundances greater than 20%, and to full recovery. Rather than resulting in a plain predator pit from direct consumption of sea lions, the age-structured sigmoid response yields a *predation-driven productivity pit* mediated by female recruitment from which the population cannot escape even at a theoretical natality of 1.0 without reduced predation pressure.

Even though our density-dependent conceptual framework with age-structured predation rates provides the most parsimonious explanation for all observed vital rate dynamics, it is important to consider that actual dynamics could be heavily influenced by other factors affecting non-specialist predators. In particular at low abundance levels, numerical consumption of sea lions may be subject to the availability of alternate prey. Considering the broad scale declines of many upper trophic marine vertebrates in the Gulf of Alaska, Aleutian Island and Bering Sea region (see introduction) the applicability of a Type III response at and above 20% abundance may not necessarily result in reduced predation at lower abundance that typically provides a refuge from predation in a predator pit scenario [52,53]. In addition, over broad periods of time such as the western Steller sea lion decline both non-predation causes of mortality and natality may vary. Within our framework predation at the levels estimated for 100% abundance alone would not initiate a decline (Fig. 3C). However, at the estimated pre-decline natality of 0.63 [21] a decrease in overall, cross-sectional survival from 85.1% to 83.1% could initiate a decline. Such a reduction in overall survival and thus the early decline could have been initiated by a comparably small reduction in carrying capacity [9,59].

Conclusions

Our data and model do not support the hypotheses derived from extant age-structured demographic models for the western Steller sea lion of recently recovered juvenile survival and depressed reproductive rates, for our study area. Instead, our data demonstrate continued low juvenile survival in the Prince William Sound/Kenai Fjords region of the Gulf of Alaska, and indirectly confirm recently published empirical studies in support of high reproductive rates. Our results on contemporary predation rates combined with a density dependent conceptual framework suggest predation on juvenile sea lions as the largest impediment to recovery of the species in the eastern Gulf of Alaska region. Our data and model do not however allow the determination of historic causes of the decline, nor of primary factors driving population trajectories outside of the study region. Nevertheless, our

conceptual framework generally suggests the distinct possibility of predation as a major component of Steller sea lion population dynamics in particular at intermediate and low abundance levels. The framework also highlights the necessity for demographic models based on age-structured census data to incorporate the differential impact of predation on multiple vital rates, in order to gain credibility. As highlighted by Boyd in 2010 [59], the applicability of extant population models fitted to census data are limited by unknown and non-stationary error structures within datasets, including population structure data. The empirical validation of the functional and numerical response applicable to Steller sea lions and their predator and the impact of the availability of alternate prey (to the sea lions' predators) thus emerge as key research objectives in particular for regions of continuing decline.

Supporting Information

Table S1 Contemporary Steller sea lion vital rate schedules for the eastern Gulf of Alaska (LHX-eGoA). The age classes listed (i) comprise months 1 through 12 for the first year, months 13 through 24 for the second year, and so forth. The survivorship schedules s_i list the proportion of animals that were alive at the beginning of each year, that survive to the end of year i. PP_i is the proportion of mortality (1-s) attributed to predation, for each year i. Mortality schedules m_i list the proportion of animals consumed by predators (p) and those that died from other causes (np) by the end of each year i. The minimum birth rate N_{min} (for definition see methods) for an equilibrium survivorship schedule is listed. Also listed are two survivorship schedules from Holmes et al. [21] for pre-decline conditions (HFYS-Pre for 1976–1982), as well for the 1998–2006 period (HFYS-06).
(DOC)

Author Contributions

Conceived and designed the experiments: MH. Performed the experiments: MH JEM. Analyzed the data: MH. Contributed reagents/materials/analysis tools: MH JEM. Wrote the paper: MH. Conducted field work: JEM MH Obtained research permits and supervised husbandry: JEM Conducted physiological control studies: JEM Revised and approved final version of paper: MH JEM.

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Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines

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The population extinction pulse we describe here shows, from a quantitative viewpoint, that Earth's sixth mass extinction is more severe than perceived when looking exclusively at species extinctions. Therefore, humanity needs to address anthropogenic population extirpation and decimation immediately. That conclusion is based on analyses of the numbers and degrees of range contraction (indicative of population shrinkage and/or population extinctions according to the International Union for Conservation of Nature) using a sample of 27,600 vertebrate species, and on a more detailed analysis documenting the population extinctions between 1900 and 2015 in 177 mammal species. We find that the rate of population loss in terrestrial vertebrates is extremely high—even in "species of low concern." In our sample, comprising nearly half of known vertebrate species, 32% (8,851/27,600) are decreasing; that is, they have decreased in population size and range. In the 177 mammals for which we have detailed data, all have lost 30% or more of their geographic ranges and more than 40% of the species have experienced severe population declines (>80% range shrinkage). Our data indicate that beyond global species extinctions Earth is experiencing a huge episode of population declines and extirpations, which will have negative cascading consequences on ecosystem functioning and services vital to sustaining civilization. We describe this as a "biological annihilation" to highlight the current magnitude of Earth's ongoing sixth major extinction event.

sixth mass extinction | population declines | population extinctions | conservation | ecosystem service

The loss of biological diversity is one of the most severe human-caused global environmental problems. Hundreds of species and myriad populations are being driven to extinction every year (1–8). From the perspective of geological time, Earth's richest biota ever is already well into a sixth mass extinction episode (9–14). Mass extinction episodes detected in the fossil record have been measured in terms of rates of global extinctions of species or higher taxa (e.g., ref. 9). For example, conservatively almost 200 species of vertebrates have gone extinct in the last 100 y. These represent the loss of about 2 species per year. Few realize, however, that if subjected to the estimated "background" or "normal" extinction rate prevailing in the last 2 million years, the 200 vertebrate species losses would have taken not a century, but up to 10,000 y to disappear, depending on the animal group analyzed (11). Considering the marine realm, specifically, only 15 animal species have been recorded as globally extinct (15), likely an underestimate, given the difficulty of accurately recording marine extinctions. Regarding global extinction of invertebrates, available information is limited and largely focused on threat level. For example, it is estimated that 42% of 3,623 terrestrial invertebrate species, and 25% of 1,306 species of marine invertebrates assessed on the International Union for Conservation of Nature (IUCN) Red List are classified as threatened with extinction (16). However, from the perspective of a human lifetime it is difficult to appreciate the current magnitude of species extinctions. A rate of two vertebrate species extinctions per year does not generate enough public concern, especially because many of those species were obscure and had limited ranges, such as the Catarina pupfish (*Megupsilon aporus*, extinct in 2014), a tiny fish from Mexico, or the Christmas Island pipistrelle (*Pipistrellus murrayi*, extinct in 2009), a bat that vanished from its namesake volcanic remnant.

Species extinctions are obviously very important in the long run, because such losses are irreversible and may have profound effects ranging from the depletion of Earth's inspirational and esthetic resources to deterioration of ecosystem function and services (e.g., refs. 17–20). The strong focus among scientists on species extinctions, however, conveys a common impression that Earth's biota is not dramatically threatened, or is just slowly entering an episode of major biodiversity loss that need not generate deep concern now (e.g., ref. 21, but see also refs. 9, 11, 22). Thus, there might be sufficient time to address the decay of biodiversity later, or to develop technologies for "deextinction"—the possibility of the latter being an especially dangerous misimpression (see ref. 23). Specifically, this approach has led to the neglect of two critical aspects of the present extinction episode: (*i*) the disappearance of populations, which essentially always precedes species extinctions, and (*ii*) the rapid decrease in numbers of individuals within some of the remaining populations. A detailed analysis of the loss of individuals and populations makes the problem much clearer and more worrisome, and highlights a whole set of parameters that are increasingly critical in considering the Anthropocene's biological extinction crisis.

Significance

The strong focus on species extinctions, a critical aspect of the contemporary pulse of biological extinction, leads to a common misimpression that Earth's biota is not immediately threatened, just slowly entering an episode of major biodiversity loss. This view overlooks the current trends of population declines and extinctions. Using a sample of 27,600 terrestrial vertebrate species, and a more detailed analysis of 177 mammal species, we show the extremely high degree of population decay in vertebrates, even in common "species of low concern." Dwindling population sizes and range shrinkages amount to a massive anthropogenic erosion of biodiversity and of the ecosystem services essential to civilization. This "biological annihilation" underlines the seriousness for humanity of Earth's ongoing sixth mass extinction event.

In the last few decades, habitat loss, overexploitation, invasive organisms, pollution, toxification, and more recently climate disruption, as well as the interactions among these factors, have led to the catastrophic declines in both the numbers and sizes of populations of both common and rare vertebrate species (24–28). For example, several species of mammals that were relatively safe one or two decades ago are now endangered. In 2016, there were only 7,000 cheetahs in existence (29) and less than 5,000 Borneo and Sumatran orangutans (*Pongo pygmaeus* and *P. abelli*, respectively) (28). Populations of African lion (*Panthera leo*) dropped 43% since 1993 (30), pangolin (*Manis* spp.) populations have been decimated (31), and populations of giraffes dropped from around 115,000 individuals thought to be conspecific in 1985, to around 97,000 representing what is now recognized to be four species (*Giraffa giraffa*, *G. tippelskirchi*, *G. reticulata*, and *G. camelopardalis*) in 2015 (32).

An important antecedent to our work (25) used the number of genetic populations per unit area and then estimated potential loss on the basis of deforestation estimates and the species–area relationship (SAR). Given the recognized limitations of the use of SAR to estimate extinctions, our work provides an approach based on reduction of species range as a proxy of population extirpation. The most recent Living Planet Index (LPI) has estimated that wildlife abundance on the planet decreased by as much as 58% between 1970 and 2012 (4). The present study is different from LPI and other related publications in several ways, including that here we use all decreasing species of vertebrates according to IUCN, mapping and comparing absolute and relative numbers of species, and focusing on population losses. Previous estimates seem validated by the data we present here on the loss of local populations and the severe decrease in the population size of many others (see also refs. 3, 4, 6–8, 26). Here we examine the magnitude of losses of populations of land vertebrate species on a global system of 10,000-km² quadrats (*Methods*). Species vary from common to rare, so that our analysis, which includes all land vertebrate species (amphibians, birds, reptiles, and mammals) deemed as "decreasing" by IUCN, provides a better estimate of population losses than using exclusively IUCN data on species at risk. Obviously, common species decreasing are not ordinarily classified as species at risk. IUCN criteria provide quantitative thresholds for population size, trend, and range size, to determine decreasing species (28, 33). We also evaluate shrinking ranges and population declines for 177 species of mammals for which data are available on geographic range shrinkage from ~1900 to 2015. We specifically focus on local extinctions by addressing the following questions: (*i*) What are the numbers and geographic distributions of decreasing terrestrial vertebrate species (i.e., experiencing population losses)? (*ii*) What are the vertebrate groups and geographic regions that have the highest numbers and proportions of decreasing species? (*iii*) What is the scale of local population declines in mammals—a proxy for other vertebrates? By addressing these questions, we conclude that anthropogenic population extinctions amount to a massive erosion of the greatest biological diversity in the history of Earth and that population losses and declines are especially important, because it is populations of organisms that primarily supply the ecosystem services so critical to humanity at local and regional levels.

Results

Patterns of Variation in Population Loss Among Vertebrates. Considering all land vertebrates, our spatially explicit analyses indicate a massive pulse of population losses, with a global epidemic of species declines. Those analyses support the view that the decay of vertebrate animal life is widespread geographically, crosses phylogenetic lineages, and involves species ranging in abundance from common to rare (Figs. 1–4). The losses, however, are not uniform: some regions exhibit higher concentrations of species with local population extinctions than others, including a strong latitudinal signal corresponding to an intertropical peak (i.e., roughly between the Tropics of Cancer and Capricorn) of number of decreasing

species, particularly strong in mammals and birds, which largely drive the overall land vertebrate pattern (Fig. 3, *Center*). Notably, some parts of the planet harbor low absolute numbers of vertebrate species undergoing decline (Figs. 2 and 3), such as those areas of low species richness located in hypercold (northernmost locations, particularly of the Western Hemisphere) and hyperarid (Saharan Africa and Central Asia) regions. However, it is instructive to examine their corresponding proportional numbers, an aspect we discuss in detail in another section below.

The number of decreasing species of all land vertebrates in each of the 10,000-km² quadrats over Earth's land surface ranges from a few to more than 365 (Fig. 2). As expected, large concentrations of decreasing vertebrate species occur in species-rich areas of moist tropical forests adjacent to mountainous regions, such as the Andes–Amazon region, the Congo basin-adjacent eastern African highlands, and the Himalayas–south Asian jungle belt. The distribution of the number of decreasing species considering vertebrate classes separately reveals notable differences. First, the maximum number of decreasing species in a 10,000-km² quadrat varies from a high value of 296 decreasing birds per quadrat, to a low maximum of 60 decreasing reptiles in a quadrat. Second, mammals and birds have relatively similar distribution patterns of

Fig. 1. Decreasing land vertebrates, as exemplified with these four species, include taxa with different conservation status (e.g., low concern, critically endangered), current geographic range (e.g., large, very restricted), and abundance (e.g., common, rare). The data on conservation status, current geographic range, and abundance are from IUCN (28). Barn swallow image courtesy of Daniel Garza Galindo (photographer).
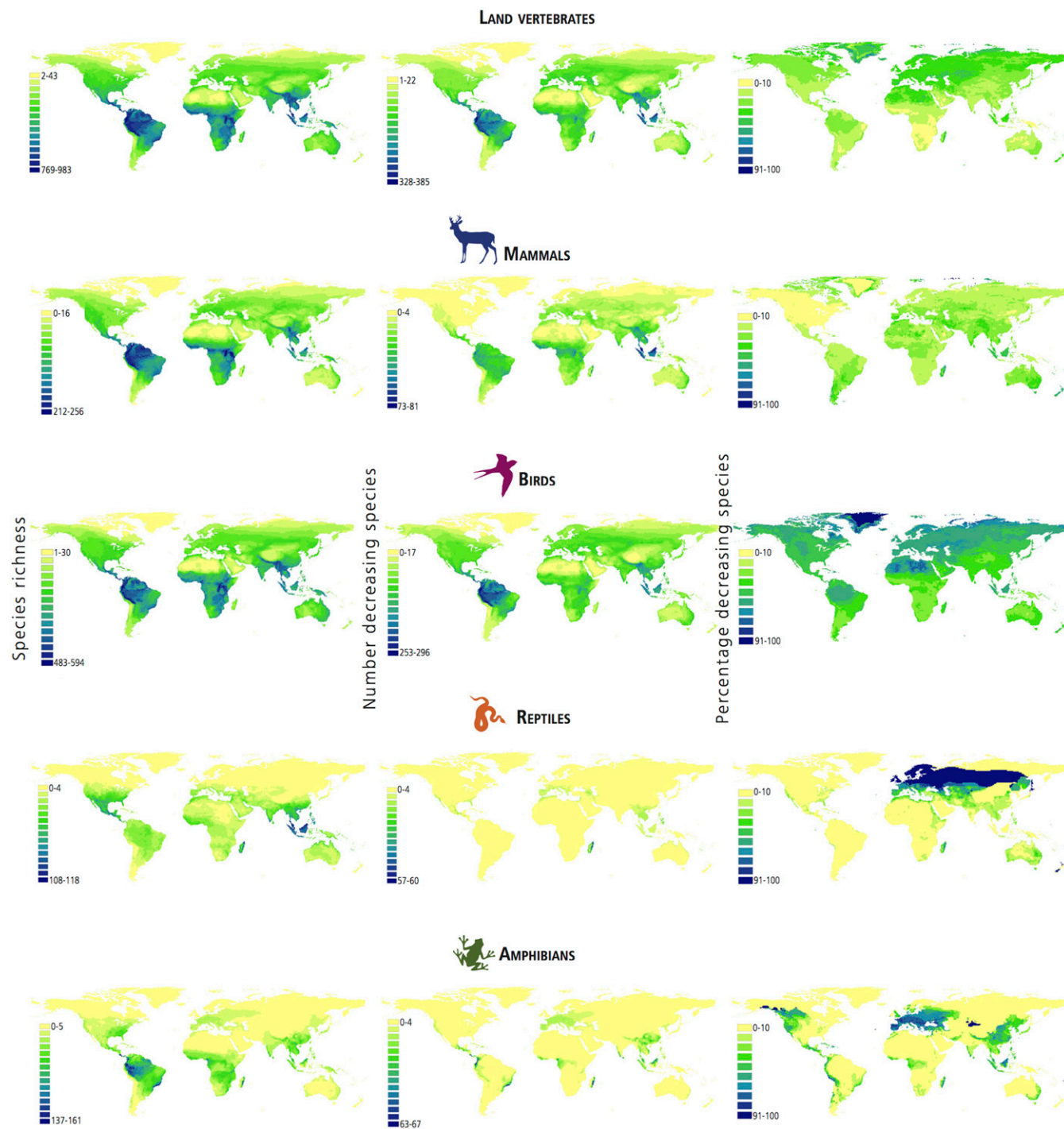
Fig. 2. Global distribution of terrestrial vertebrate species according to IUCN (28). (*Left*) Global distribution of species richness as indicated by number of species in each 10,000-km² quadrat. (*Center*) Absolute number of decreasing species per quadrat. (*Right*) Percentage of species that are suffering population losses in relation to total species richness per quadrat. The maps highlight that regions of known high species richness harbor large absolute numbers of species experiencing high levels of decline and population loss (particularly evident in the Amazon, the central African region, and south/southeast Asia), whereas the proportion of decreasing species per quadrat shows a strong high-latitude and Saharan Africa signal. In addition, there are several centers of population decline in both absolute and relative terms (Borneo, for example).

decreasing species, except that birds have more decreasing species in the temperate zones. Third, mammals and birds have patterns of decreasing species quite distinct from those of reptiles and amphibians (Figs. 2 and 3), given that the latter are rarer in the northern and southern temperate and subpolar regions (both are essentially absent from the Arctic and are missing from the Antarctic). Fourth, reptiles

and amphibians clearly differ from each other in regions where decreasing species are concentrated. For example, there are more decreasing reptiles in the Eurasian and African continents, and more decreasing amphibians in the Americas.

There is also great variation in the total population size and geographic ranges among individual species. Although there is no
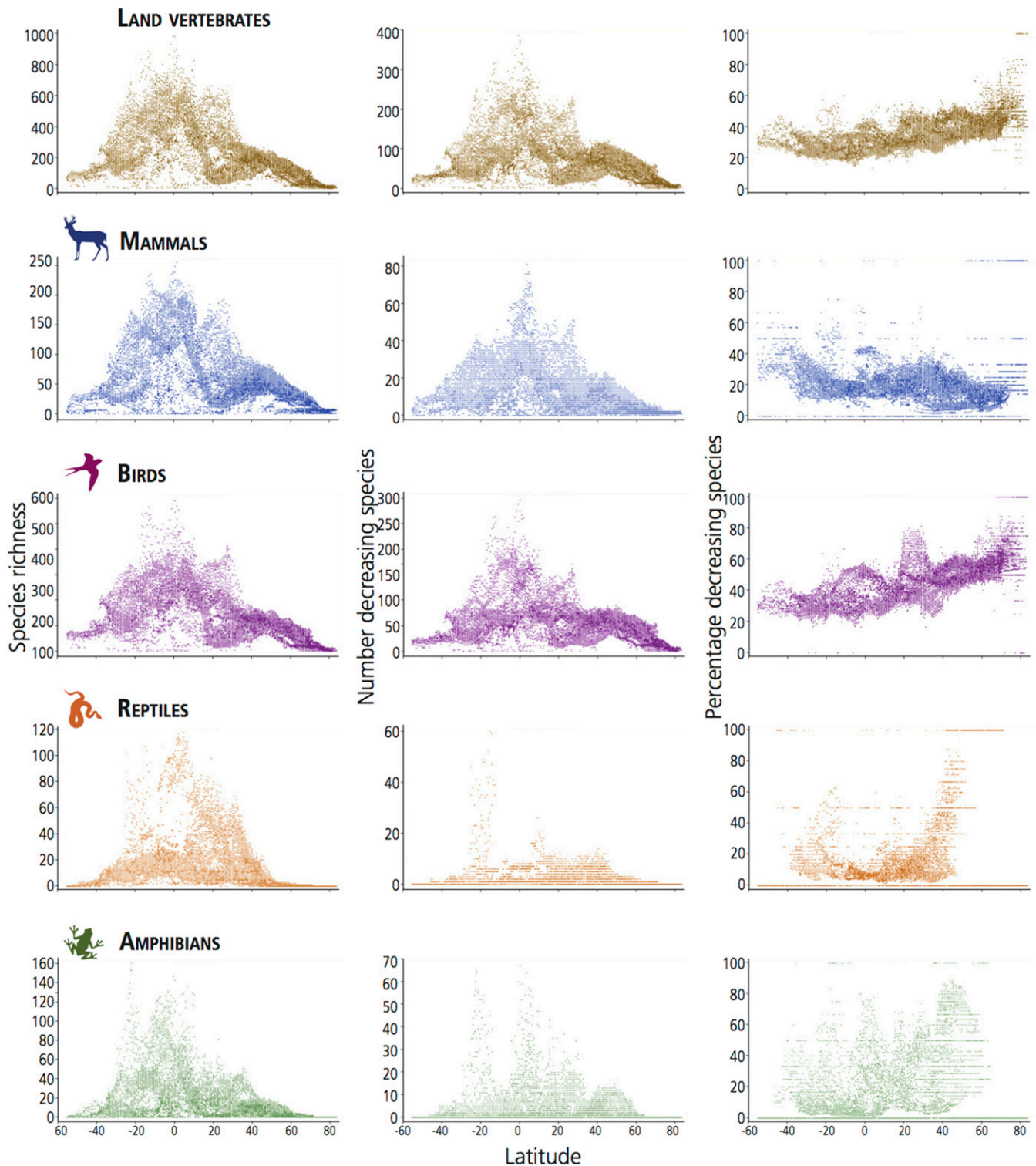
Fig. 3. Latitudinal distribution of species richness (*Left*), decreasing species (*Center*), and the percentage of species (*Right*) that are suffering population losses in relation to total species richness, in each 10,000-km² quadrat. Patterns of species richness in relation to latitude are similar in all vertebrates, although there are more species per quadrat in birds and mammals and, as expected, a scarcity of reptiles and amphibians at high latitudes. The patterns of number of species with decreasing populations indicate that regions with high species richness also have high numbers of decreasing species, but the percentage of decreasing species in relation to species richness shows contrasting patterns between mammals and birds compared with reptiles and amphibians. In mammals and birds, the percentage of decreasing species is relatively similar in regions with low and high species richness. In contrast, there are proportionally more decreasing species of reptiles and amphibians in regions with low species richness.

accurate information on population size for most taxa, whatever is available indicates that the total population size in species with

decreasing populations varies from fewer than 100 individuals in critically endangered species such as the Hainan black-crested
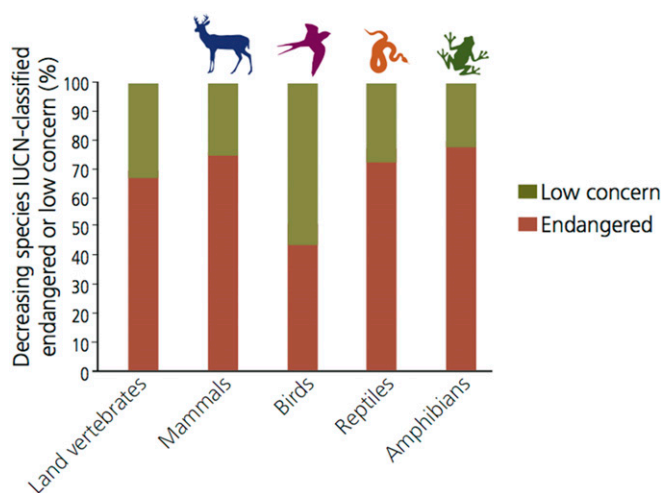
Fig. 4. The percentage of decreasing species classified by IUCN as "endangered" (including "critically endangered," "endangered," "vulnerable," and "near-threatened") or "low concern" (including "low concern" and "data-deficient") in terrestrial vertebrates. This figure emphasizes that even species that have not yet been classified as endangered (roughly 30% in the case of all vertebrates) are declining. This situation is exacerbated in the case of birds, for which close to 55% of the decreasing species are still classified as "low concern."

gibbon (*Nomascus hainanus*), to many millions of individuals in decreasing common species such as the barn swallow (*Hirundo rustica*). Similarly, the smallest ranges (i.e., <1 km^2) are seen in species such as the Carrizal seedeater (*Amaurospiza carrizalensis*) from Venezuela and Herrera's false coral snake (*Lampropeltis herrerae*) from Mexico, both denizens of tiny islands. The largest ranges are hundreds of thousands of square kilometers, as in the bush dog (*Speothos venaticus*) from South America and the common lizard (*Zootoca vivipara*) from Eurasia. The sum of the 10,000-km^2 quadrats representing the current ranges of the 8,851 decreasing vertebrate species is 1,350,876 quadrats. A highly conservative estimate would indicate a similar number of local populations facing extinction. This is, of course, a very rough estimate of the total number of populations, as the number of populations of a decreasing species in each quadrat largely depends, aside from suitable habitat distribution within the quadrat, on animal body mass and trophic position (e.g., ref. 34). The assumption of one population per 10,000 km^2 might seem very conservative, as this area could accommodate many populations of small animals (e.g., 0.1-kg rodents), most of which could have been extirpated. However, 10,000 km^2 may not be sufficient for, or can barely accommodate a viable population of large carnivores (say a 330-kg Siberian tiger; ref. 34). Nonetheless, our results provide evidence of the extremely large numbers of vertebrate populations facing extinction, compared with the number of species.

Proportion of Vertebrate Species Decreasing. The proportion of decreasing vertebrates shows that there are areas across the planet with high concentrations of decreasing species in all vertebrates and regions with high proportions of decreasing species of a particular group (Figs. 2, 3, and 5). For example, in mammals, the highest percentage of decreasing species is concentrated in tropical regions, mostly in the Neotropics and Southeast Asia, whereas in reptiles, the proportional decline concentrates almost exclusively in Madagascar. Decreasing amphibians are prominent in Mexico, Central America, the northern Andes, and Brazil's Atlantic forest in the Americas; West Africa and Madagascar in Africa; and India and Southeast Asia, including Indonesia and Philippines in Asia–Southeast Asia. Finally, decreasing species of birds are found over large regions of all continents (Fig. 2).

Roughly a third (8,851/27,600) of all land vertebrate species examined are experiencing declines and local population losses of a considerable magnitude (Figs. 2–4). Such proportion of decreasing species varies, depending on the taxonomic group, from 30% or more in the case of mammals, birds, and reptiles, to 15% in the case of amphibians. Furthermore, of the decreasing species, many are now considered endangered (Fig. 4). Beyond that, roughly 30% of all decreasing species are still sufficiently common that they are considered of "low concern" by IUCN, rather than "endangered." That so many common species are decreasing is a strong sign of the seriousness of the overall contemporary biological extinction episode.

In our 10,000-km^2 quadrats, the proportion of decreasing species ranges from less than 10% to more than 50% (Fig. 2). The geographic distributions of absolute (i.e., number) and relative (i.e., percentage) of decreasing species is contrasting. Whereas tropical regions have larger numbers of decreasing species, as expected, given their higher species richness, their corresponding proportions are relatively low. In contrast, temperate regions tend to have similar or higher proportions of decreasing species, a trend dramatically prominent in the case of reptiles.

Local Population Extinctions in Mammals. Our most detailed data allow comparison of historic and present geographic range of a sample of 177 mammal species (Figs. 5 and 6). Most of the 177 mammal species we sampled have lost more than 40% of their geographic ranges in historic times, and almost half have lost more than 80% of their ranges in the period ~1900–2015. At the continental and subcontinental level, some patterns become evident (Fig. 5). The predominant category of range contraction is ≥80% in Africa (56% of the sampled mammal species), Asia (75% of the species), Australia (60% of the species), and Europe (40% of the species). In the Americas, range contractions are less marked but still considerable: 22% of the species in North America and 17% of the species in South America have experienced range contractions of at least 80%. Nevertheless, 50% of the species in North America and 28% of the species in South America have experienced a range contraction of 41% or more.

The comparison of the 1900–2015 geographic ranges showed that the 177 species of mammals have disappeared from 58,000 grid cells. On the assumption that on average each of the 10,000-km^2 occupied quadrats held a single population of the species found within it, this implies that roughly 58,000 populations of the 177 mammals we examined have gone extinct. Consider the following emblematic cases: The lion (*Panthera leo*) was historically distributed over most of Africa, southern Europe, and the Middle
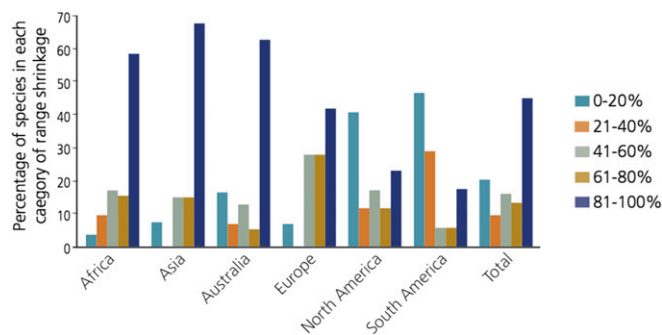
Fig. 5. The percentage of species of land mammals from five major continents/subcontinents and the entire globe undergoing different degrees (in percentage) of decline in the period ~1900–2015. Considering the sampled species globally, 56% of them have lost more than 60% of their range, a pattern that is generally consistent in Africa, Asia, Australia, and Europe, whereas in South America and North America, 35–40% of the species have experienced range contractions of only 20% or less. (See text for details.)
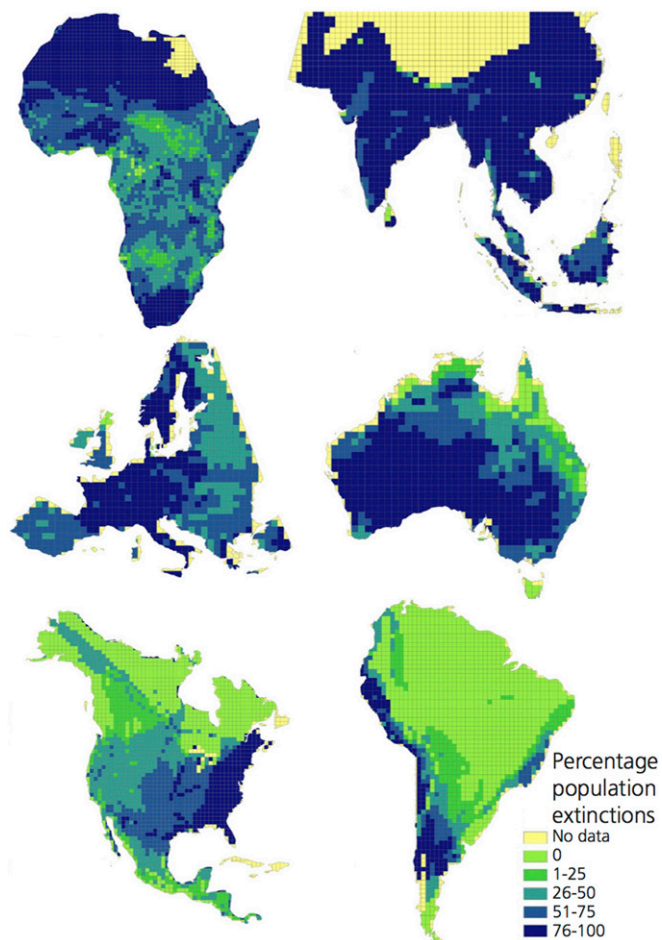
Fig. 6. Percentage of local population extinction in 177 species of mammals in 1° × 1° quadrats, as an indication of the severity of the mass extinction crises. The maps were generated by comparing historic and current geographic ranges (49) (*SI Appendix, SI Methods*). Note that large regions in all continents have lost 50% or more of the populations of the evaluated mammals. Because of the small sample size, biased to large mammal species, this figure can only be used to visualize likely trends in population losses.

Percentage population extinctions
- No data
- 0
- 1-25
- 26-50
- 51-75
- 76-100

East, all the way to northwestern India (*SI Appendix*, Fig. S1). It is now confined to scattered populations in sub-Saharan Africa and a remnant population in the Gir forest of India. The vast majority of lion populations are gone. In its African stronghold, it historically occupied roughly two thousand 10,000-km² cells, and now it is reduced to some 600 cells. Other species, such as the mountain lion (*Puma concolor*), are known to be doing better. The mountain lion has lost some of its local populations in North America, but has not suffered such disastrous losses as its Old World relative, adapting relatively well to human-dominated landscapes, and it is still found across 85% of its historic range.

Clearly, the extinction of mammal populations, although varying from species to species, has been a global phenomenon (Fig. 6). Strikingly, the predominant color code in the mammalian map is that of 70% or more of population losses, with the exception of some areas of South America and high latitudes of North America. Particularly hard hit have been the mammals of south and southeast Asia, where all of the large-bodied species of mammals analyzed have lost more than 80% of their geographic ranges. The Cape and Sahara regions in Africa, central Australia, the eastern United States, and the Atlantic forest in South America have also suffered severely from population extinctions.

Discussion

It has recently been shown, using conservative estimates of current and background species extinction rates, that Earth is now in a period of mass global species extinction for vertebrate animals (11). But the true extent of this mass extinction has been underestimated, because of the emphasis on species extinction. This underestimate largely traces to overlooking the accelerating extinction of populations. Whereas scientists have known for a long time that several relatively well-studied species have undergone major contraction of their ranges, experienced considerable population decreases, and suffered many population extinctions, the global extent of population shrinkage and extirpation has previously not been recognized and quantified.

In addition, some studies document that invertebrates and plants are suffering massive losses of populations and species (35–38). Here we extend investigation of mass extinction to terrestrial vertebrate population decreases and losses, and give estimates of the number of their species with decreasing populations. The accuracy of the estimates is strongly dependent on an unknown parameter, namely, the actual average area occupied by a vertebrate population (e.g., refs. 35, 39–41). However, even if a population would, on average, occupy an area five times larger than what we have used here (i.e., 50,000 km²) there would still be hundreds of thousands of populations that have suffered extinction in the past few centuries. On the other hand, most vertebrates (~70%) are small species of mammals, birds, reptiles, and amphibians. If, on average, they have one population every 10 km² then vertebrates would have suffered more than a billion population extinctions.

Our results show that population extinction in land vertebrates is geographically omnipresent, but with notable prominence in tropical, species-rich regions. It is interesting, however, that when population extinctions are evaluated as the percentage of total species richness, temperate regions, with their typical low species diversity, show higher proportions of population loss.

There are some illustrative qualitative examples of population decreases and their consequences within terrestrial and marine vertebrates, but ours is an attempt at a quantitative evaluation of global trends in population extinctions. Recent reviews indicate that species extinctions, population decreases, and range contraction (implying population extinctions) among terrestrial invertebrates and plants are as severe as among vertebrates (e.g., refs. 35–38). For example, long-term monitoring of insect populations in the United Kingdom shows that 30–60% of species per taxonomic order have contracting ranges (36). The situation in plants has been less evaluated; thus it is difficult to compare them with animals, but there is little reason to believe that the extinction situation in plants is dramatically different (37). Furthermore, research shows that the loss of animal populations indirectly leads to changes in plant communities (20, 37, 39), frequently causing the reduction of local species richness and dominance of a few plant taxa that either experience "ecological release" in response to decreasing herbivore pressures (42, 43), and/or experience population reductions due to the decline of animals responsible for pollination or dispersal (e.g., refs. 2–3, 20). The status of biodiversity among microorganisms is too poorly known to permit us to make any comparison and generalizations about the current pulse of extinctions, although some recent research has unraveled feedbacks between local large herbivore defaunation and mycorrhizal richness (44, 45). Given what we know about genetic population differentiation, it is expected that the range contractions and declines we document here imply a considerable loss of intraspecific genetic diversity (23) but this is, clearly, an aspect that warrants further investigation.

In sum, by losing populations (and species) of vertebrates, we are losing intricate ecological networks involving animals, plants, and microorganisms (e.g., refs. 2, 8, 18, 45, 46). We are also losing pools of genetic information that may prove vital to species' evolutionary adjustment and survival in a rapidly changing global environment.

This suggests that, even if there was not ample sign that the crisis extends far beyond that group of animals, today's planetary defaunation of vertebrates will itself promote cascading catastrophic effects on ecosystems, worsening the annihilation of nature (2, 3, 46). Thus, while the biosphere is undergoing mass species extinction (11), it is also being ravaged by a much more serious and rapid wave of population declines and extinctions. In combination, these assaults are causing a vast reduction of the fauna and flora of our planet. The resulting biological annihilation obviously will also have serious ecological, economic, and social consequences (46). Humanity will eventually pay a very high price for the decimation of the only assemblage of life that we know of in the universe.

Conclusion

Population extinctions today are orders of magnitude more frequent than species extinctions. Population extinctions, however, are a prelude to species extinctions, so Earth's sixth mass extinction episode has proceeded further than most assume. The massive loss of populations is already damaging the services ecosystems provide to civilization. When considering this frightening assault on the foundations of human civilization, one must never forget that Earth's capacity to support life, including human life, has been shaped by life itself (47). When public mention is made of the extinction crisis, it usually focuses on a few animal species (hundreds out of millions) known to have gone extinct, and projecting many more extinctions in the future. But a glance at our maps presents a much more realistic picture: they suggest that as much as 50% of the number of animal individuals that once shared Earth with us are already gone, as are billions of populations. Furthermore, our analysis is conservative, given the increasing trajectories of the drivers of extinction and their synergistic effects. Future losses easily may amount to a further rapid defaunation of the globe and comparable losses in the diversity of plants (36), including the local (and eventually global) defaunation-driven coextinction of plants (3, 20). The likelihood of this rapid defaunation lies in the proximate causes of population extinctions: habitat conversion, climate disruption, overexploitation, toxification, species invasions, disease, and (potentially) large-scale nuclear war—all tied to one another in complex patterns and usually reinforcing each other's impacts. Much less frequently mentioned are, however, the ultimate drivers of those immediate causes of biotic destruction, namely, human overpopulation and continued population growth, and overconsumption, especially by the rich. These drivers, all of which trace to the fiction that perpetual growth can occur on a finite planet, are themselves increasing rapidly. Thus, we emphasize that the sixth mass extinction is already here and the window for effective action is very short, probably two or three decades at most

(11, 48). All signs point to ever more powerful assaults on biodiversity in the next two decades, painting a dismal picture of the future of life, including human life.

Methods

For full methods, please see *SI Appendix*. We determined the number of decreasing vertebrate species using the IUCN (28) Red List of Threatened Species. In the IUCN, species are classified as decreasing, stable, or increasing (see also ref. 33). Either range contraction (population extinction) or reduction in numbers in extant populations determines whether a species is decreasing. We used the IUCN maps of terrestrial vertebrates (i.e., mammals, birds, reptiles, and amphibians) to create the global maps of number of species (richness) and of decreasing species, and percentage of decreasing species in relation to total species richness. The distribution of all of the species was superimposed in a 22,000 grid of 10,000-km^2 quadrats covering the continental lands. For the grid, a Lambert azimuthal equal-area projection was used (see ref. 49 for details of the projection methods). In our analyses a critical issue is how grid squares and populations correspond. This is a very difficult problem that varies with definitions of species. (In this paper, we stick with the classic biological definition of species.) The number of populations also varies from species to species; for example, a highly phylopatric species would have more populations per square than a very vagile species, and species with different mating systems would have different estimates of numbers of Mendelian populations, and these would not be the same as estimates of number of demographic units (50). For the purposes of understanding the annihilation, these differences are not critical. For example, if we have lost 90% of the lion's geographic range, whether this amounts to 10,000 demographic units or 4,000 Mendelian populations is trivial in the present context. It would be extremely useful if we had much more information on population structure for all vertebrates, but this is a major, pending agenda.

The population extinction analysis was conducted on 177 mammalian species occurring on five continents. Specifically, we analyzed 54 species in Africa, 14 in Asia, 57 in Australia, 15 in Europe, and 35 in America. The historical distribution was gathered from specialized literature (see details in ref. 26) and the current distribution from IUCN (28). Historic and current ranges were digitized as geographic information system polygons and elaborated in ArcGis 10.1 (51). For each species, we calculated the area of the historical and present distribution (in square kilometers) to estimate the percentage of lost area and the percentage of area where the species are extant. A caveat of these estimates regards how representative the sample of 177 species is. We recognize a bias in that the data include a large number of medium- and large-sized species, for which the best information is available. However, given that such medium and large species are the most seriously threatened by the predominant proximate drivers of defaunation (2, 3), the likely bias against small-sized species should not affect our overall interpretation of results.

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More than 75 percent decline over 27 years in total flying insect biomass in protected areas

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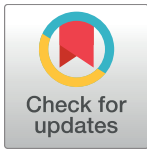
Abstract

Global declines in insects have sparked wide interest among scientists, politicians, and the general public. Loss of insect diversity and abundance is expected to provoke cascading effects on food webs and to jeopardize ecosystem services. Our understanding of the extent and underlying causes of this decline is based on the abundance of single species or taxonomic groups only, rather than changes in insect biomass which is more relevant for ecological functioning. Here, we used a standardized protocol to measure total insect biomass using Malaise traps, deployed over 27 years in 63 nature protection areas in Germany (96 unique location-year combinations) to infer on the status and trend of local entomofauna. Our analysis estimates a seasonal decline of 76%, and mid-summer decline of 82% in flying insect biomass over the 27 years of study. We show that this decline is apparent regardless of habitat type, while changes in weather, land use, and habitat characteristics cannot explain this overall decline. This yet unrecognized loss of insect biomass must be taken into account in evaluating declines in abundance of species depending on insects as a food source, and ecosystem functioning in the European landscape.

Introduction

Loss of insects is certain to have adverse effects on ecosystem functioning, as insects play a central role in a variety of processes, including pollination [1, 2], herbivory and detrivory [3, 4], nutrient cycling [4] and providing a food source for higher trophic levels such as birds, mammals and amphibians. For example, 80% of wild plants are estimated to depend on insects for pollination [2], while 60% of birds rely on insects as a food source [5]. The ecosystem services provided by wild insects have been estimated at $57 billion annually in the USA [6]. Clearly, preserving insect abundance and diversity should constitute a prime conservation priority.

Current data suggest an overall pattern of decline in insect diversity and abundance. For example, populations of European grassland butterflies are estimated to have declined by 50% in abundance between 1990 and 2011 [7]. Data for other well-studied taxa such as bees [8–14]

and moths [15–18] suggest the same trend. Climate change, habitat loss and fragmentation, and deterioration of habitat quality have been proposed as some of the prime suspects responsible for the decline [9–11, 13, 18–22]. However, the number of studies on insect trends with sufficient replication and spatial coverage are limited [10, 23–25] and restricted to certain well-studied taxa. Declines of individual species or taxa (e.g. [7, 26]) may not reflect the general state of local entomofauna [27]. The total insect biomass would then be a better metric for the status of insects as a group and its contribution to ecosystem functioning, but very few studies have monitored insect biomass over an extensive period of time [28]. Hence, to what extent total insect biomass has declined, and the relative contribution of each proposed factor to the decline, remain unresolved yet highly relevant questions for ecosystem ecology and conservation.

Here, we investigate total aerial insect biomass between 1989 and 2016 across 96 unique location-year combinations in Germany, representative of Western European low-altitude nature protection areas embedded in a human-dominated landscape (S1 Fig). In all years we sampled insects throughout the season (March through October), based on a standardized sampling scheme using Malaise traps. We investigated rate of decline in insect biomass, and examined how factors such as weather, habitat and land use variables influenced the declines. Knowledge on the state of insect biomass, and it's direction over time, are of broad importance to ecology and conservation, but historical data on insect biomass have been lacking. Our study makes a first step into filling this gap, and provides information that is vital for the assessment of biodiversity conservation and ecosystem health in agricultural landscapes.

Materials and methods

Data

Biomass data. Biomass data were collected and archived using a standardized protocol across 63 unique locations between 1989 and 2016 (resulting in 96 unique location-year combinations) by the Entomological Society Krefeld. The standardized protocol of collection has been originally designed with the idea of integrating quantitative aspects of insects in the status assessment of the protected areas, and to construct a long-term archive in order to preserve (identified and not-identified) specimens of local diversity for future studies. In the present study, we consider the total biomass of flying insects to assess the state of local entomofauna as a group.

All trap locations were situated in protected areas, but with varying protection status: 37 locations are within Natura2000 sites, seven locations within designated Nature reserves, nine locations within Protected Landscape Areas (with funded conservation measures), six locations within Water Protection Zones, and four locations of protected habitat managed by Regional Associations. For all location permits have been obtained by the relevant authorities, as listed in the S1 Appendix. In our data, traps located in nutrient-poor heathlands, sandy grasslands, and dune habitats provide lower quantities of biomass as compared to nutrient nutrient-rich grasslands, margins and wastelands. As we were interested in whether the declines interact with local productivity, traps locations were pooled into 3 distinct habitat clusters, namely: nutrient-poor heathlands, sandy grassland, and dunes (habitat cluster 1, n = 19 locations, Fig 1A), nutrient-rich grasslands, margins and wasteland (habitat cluster 2, n = 41 locations, Fig 1B) and a third habitat cluster that included pioneer and shrub communities (n = 3 locations).

Most locations (59%, n = 37) were sampled in only one year, 20 locations in two years, five locations in three years, and one in four years, yielding in total 96 unique location-year combinations of measurements of seasonal total flying insect biomass. Our data do not represent

Fig 1. Examples of operating malaise traps in protected areas in western Germany, in habitat cluster 1 (A) and cluster 2 (B) (see [Materials and methods](#)).

Table 1. Overview of malaise-trap samples sizes. For each year, the number of locations sampled, the number of location re-sampled, total number of samples, as well as mean and standard deviation of exposure time at the trap locations (in days) are presented.

Year	Number of locations	Number of locations sampled previously	Number of Samples	Mean exposure time	St. Dev exposure time
1989	8	0	162	146.62	12.81
1990	2	0	62	228.50	34.65
1991	1	0	10	146.00	
1992	4	0	54	118.75	15.50
1993	4	0	39	109.50	59.74
1994	4	0	60	170.75	72.83
1995	2	0	41	144.00	93.34
1997	1	0	20	162.00	
1999	2	0	56	196.00	0.00
2000	2	1	47	174.00	11.31
2001	3	2	81	190.00	0.00
2003	3	1	80	201.00	7.81
2004	2	0	48	200.00	5.66
2005	4	0	70	198.75	30.53
2006	2	0	26	188.00	0.00
2007	2	0	15	192.00	0.00
2008	2	0	24	162.00	0.00
2009	4	0	23	120.50	2.89
2010	2	0	12	85.00	0.00
2011	1	0	4	68.00	
2012	2	0	23	158.50	4.95
2013	8	2	126	175.50	21.71
2014	23	19	348	212.74	11.21
2015	1	1	10	224.00	
2016	7	7	62	190.86	12.56

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longitudinal records at single sites, suitable to derive location specific trends (e.g. [28]). Prolonged trapping across years is in the present context (protected areas) deemed undesirable, as the sampling process itself can negatively impact local insect stocks. However, the data do permit an analysis at a higher spatial level, i.e. by treating seasonal insect biomass profiles as random samples of the state of entomofauna in protected areas in western Germany.

Malaise traps were deployed through the spring, summer and early autumn. They operated continuously (day and night), and catches were emptied at regular intervals, on average every 11.2 days (sd = 6.3). We collected in total 1503 trap samples, with an average of 16 (4–35) successive catches per location-year combination (Table 1). Between 1989 and 2016, a total of 53.54kg of invertebrates have been collected and stored, over a total trap exposure period of 16908 days, within an average of 176 exposure days per location-year combination. Malaise traps are known to collect a much wider diversity of insect species (e.g. [29–31]) as compared to suction traps (e.g. [28]) and are therefore considered superior as a method of collecting flying insects. On the basis of partial assessments, we can assume that the total number of insects included in 53.54 kg biomass represents millions of individuals.

The sampling was standardized in terms of trap construction, size and design (identical parts), colors, type of netting and ground sealing, trap orientation in the field as well as slope at the trap location. Hence none of the traps differed in any of these field aspects. Our trap model was similar to the bi-colored malaise trap model by Henry Townes [32, 33]. The traps,

collecting design, and accompanying methods of biomass measurement as designed and applied by the Entomological Society Krefeld are described elsewhere [34–36] and in S2 Appendix.

Trap catches were stored in 80% ethanol solution, prior to weighing, and total insect biomass of each catch (bottle) was obtained based on a standardized measurement protocol by first subtracting fluid content. In order to optimally preserve samples for future species determination, the insects were weighed in an alcohol-wet state. First, the alcohol concentration in the vessels was stabilized to 80%, while this concentration was controlled with an areometer over a period of at least two days. In order to obtain biomass per sample with sufficient accuracy and comparability, the measuring process was fixed using a standardized protocol [34]. For this purpose the insects of a sample were poured onto a stainless steel sieve (10cm diameter) of 0.8 mm mesh width. This sieve is placed slightly obliquely (30 degrees) over a glass vessel. The skew position accelerates the first runoff of alcohol and thus the whole measuring procedure. The drop sequence is observed with a stopwatch. When the time between two drops has reached 10 seconds for the first time, the weighing process is performed with a laboratory scale. For the determination of the biomass, precision scales and analytical scales from Mettler company were used with an accuracy of at least 0.1g and controlled with calibrated test weights at the beginning of a new weighing series. In a series of 84 weightings of four different samples repeating this measurement procedure, an average deviation from the mean value of the measurement results of 0.4 percent was observed (unpublished results).

Weather data. Climate change is a well-known factor responsible for insect declines [15, 18, 21, 37]. To test if weather variation could explain the observed decline, we included mean daily temperature, precipitation and wind speed in our analysis, integrating data from 169 weather stations [38] located within 100km to the trap locations. We examined temporal trends in each weather variable over the course of the study period to assess changes in climatic conditions, as a plausible explanation for insect decline. Estimates of each weather variable at the trap locations were obtained by interpolation of each variable from the 169 climate stations.

We initially considered mean daily air temperature, precipitation, cloud cover, relative air moisture content, wind speed, and sunshine duration. However, only temperature, precipitation and wind speed were retained for analysis, as the other variables were significantly correlated with the selected variables [R(temperature, cover) = −43.2%, R(temperature, sunshine) = 53.4%, R(precipitation, moisture) = −47.3%] and because we wanted to keep the number of covariates as low as possible. Additionally, we calculated the number of frost days and the sum of precipitation in the months November- February preceding a sampling season. We used spatio-temporal geostatistical models [39, 40] to predict daily values for each weather variable to each trap location. Amongst other methods, the geostatistical approach is considered a superior interpolation method in order to derive weather variables to trap locations [41]. Uncertainty in interpolated variables such as wind speed is usually associated with altitude differences. However, as our trap locations are all situated in lowland areas with little altitude variation, we do not expect a large error in our interpolations at trap locations.

We decomposed the daily values of each weather variable into a long-term average trend (between years), a mean seasonal trend, and a yearly seasonal anomaly component (S2 Fig), modeled using regression splines [42] while controlling for altitude of weather stations. The remaining residual daily values of each station were further modeled using a spatio-temporal covariance structure. For example, temperature T, on given day t, of a given year k at a given trap location s is modeled as:

$$T(t, s, k) = f_k(k) + f_t(t) + r(k, t) + a \times h + C_{s,t} \qquad (1)$$

where $f_k(k)$ is the long-term trend over the years (a thin plate regression spline), $f_t(t)$ the mean seasonal trend within years (a penalized cyclic cubic regression spline), $r(k, t)$ the mean residual seasonal component, which measures annual anomaly in mean daily values across selected stations, and a is the linear coefficient for the altitude h effect. The spatio-temporal covariance structure $C_{s, t}$, fitted independently to the residuals of each weather variable model, allowed us to deal with lack of independence between daily weather data within and between stations, as well as to interpolate to trap locations using kriging. Altitude of trap locations was extracted from a digital elevation models at 90m resolution [43].

Land use data. Land use variables (and changes therein) were derived from aerial photographs [44] taken within two distinct time periods (between 1989–1994, and between 2012–2015), and allowed us to characterize land use composition at surroundings of the traps, as well as changes over time. We distinguished cover of forests, agricultural areas, natural grassland, and surface water. For each trap location, aerial photographs were manually processed, polygons extracted and categorized, and their surface area calculated with a radius of 200 meter. Preliminary analysis of the relationship between log biomass and landuse variables, on a subset of the trap locations, indicated that land use elements at 200m radius better predicted insect biomass than elements at 500 and 1000m radius, similar to findings elsewhere for wild bees [45]. Land use variables were measured at a coarse temporal resolution, but fortunately cover the temporal span of insect sampling. To link the cover of a given land use variable to the insect biomass samples in a particular year, we interpolated coverage between the two time points to the year of insect sampling using generalized linear models with a binomial error distribution, a logit link, and an estimated dispersion parameter. Mean distributions of land use at each of the two time points are depicted in S3A & S3B Fig.

Habitat data. Plant inventories were conducted in the immediate surroundings (within 50m) of the trap, in the same season of insect sampling. These data permitted the assessment of plant species richness (numbers of herbs, shrubs and trees) and environmental conditions based on average Ellenberg values [46–48], as well as changes therein over time. Each Ellenberg indicator (we considered nitrogen, pH, light, temperature and moisture) was averaged over all species for each location-year combination. We examined annual trends in each of the above-mentioned variables in order to uncover potential structural changes in habitat characteristics over time. Species richness was analyzed using mixed-effects generalized linear models [49] with a random intercept for trap location and assuming a Poisson distribution for species richness, and a normal distribution for mean Ellenberg indicator values. Although a Poisson distribution fitted tree and shrub species adequately, (residual deviance/ degree of freedom = 0.94 and 1.04 respectively), severe overdispersion was found for herb species richness (residual deviance/ degree of freedom = 2.16). Trend coefficients of richness over time between a Poisson mixed effects model and a negative binomial model were comparable but differed in magnitude (Poisson GLMM: −0.034 (se = 0.003), vs NB GLMM −0.027 (se = 0.006)). Although the fit is not perfect in the case of herb richness, we believe our trend adequately describes direction of change over time. Mean changes in plant species richness are depicted in S3C Fig.

Insect biomass model

The temporal resolution of the trap samples (accumulated over several days) is not directly compatible with the temporal distribution of the weather data (daily values). Additionally, variable exposure intervals between trap samples is expected to induce variation in trapped biomass between samples, and hence induce heteroscedasticity. Furthermore, biomass data can numerically only be positive on the real line, and we require a model to reflect this property of

the data. Because of the unequal exposure intervals however, log-transforming the response would be inappropriate, because we require the sum of daily values after exponentiation, rather that the exponent of the sum of log-daily biomass values. In order to indirectly relate biomass to daily weather variables, to account for the variation in time exposure intervals over which biomass was accumulated in the samples, and to respect the non-negative nature of our data, we modeled the biomass of each catch as the sum of the expected (but unobserved) latent daily biomass. The mass m of each sample j, at site s in year k, is assumed to be distributed normally about the sum of the latent expected daily mass ($z_{t, s, k}$), with variance σ_j^2:

$$m_{j,s,k} \sim N(\mu_{j,s,k}, \sigma_j^2) \tag{2}$$

subject to $\mu_{j,s,k} = \sum_{t=\tau_1(j)}^{\tau_2(j)} z_{t,s,k}$ where τ_1 and τ_2 mark the exposure interval (in days) of biomass collection of each sample j. The latent daily biomass itself is represented by a log normal distribution, in which coefficients for covariates, random effects and residual variance are all represented on the log scale. In turn, daily biomass is modeled as

$$z_{t,s,k} = e^{y_{t,s,k}} \tag{3}$$

$$y_{t,s,k} = c + log(\lambda)k + \mathbf{X}\beta_x + u_s \tag{4}$$

where c is a global intercept, $\mathbf{X}$ a design matrix of dimensions n×p (number of samples × number of covariates; see Model analysis below), β_x the corresponding vector of coefficients that measure the weather, habitat and land use effects, and $log(\lambda)$ a mean annual population growth rate parameter. The random term (u_s) denotes the location-specific random effect assumed to be distributed normally about zero $u_s \sim N(0, \sigma_{site}^2)$. The exponentiation of the right hand side of Eq (3) ensures expected values to be positive.

The expected residual variance of each sample σ_j^2, is expressed as the sum of variances of daily biomass values ($\sigma_{t,s,k}^2$).

$$\sigma_j^2 = \sum_{t=\tau_1(j)}^{\tau_2(j)} \sigma_{t,s,k}^2 \tag{5}$$

The variances of daily biomass should respect the non-negative nature of the data as well. Additionally, we are interested in being able to compare the residual variance with the random effects variance, and this requires them to be on the same scale. Therefore, we expressed the variance of the daily biomass as a function of the variance of the logarithm of the daily biomass. Using the method of moments:

$$\sigma_{t,s,k}^2 = e^{2y_{t,s,k}+v}(e^v - 1) \tag{6}$$

where v represents the residual variance of daily log-biomass.

Analysis

We developed a series of models each consisting of a set of explanatory variables that measure aspects of climate, land use and local habitat characteristics. Significant explanatory variables in these models were combined into a final model, which was then reduced to exclude insignificant effects. An overview of which covariates were included in each model is given in Table 2.

Weather effects explored were daily temperature, precipitation and wind speed, as well as the number of frost days and sum of precipitation in the preceding winter. Habitat effects explored tree and herb species richness, as well as average Ellenberg values for nitrogen, pH,

Table 2. Overview of covariates included in each of the seven models. The year covariate yields the annual trend coefficient.

Covariate class	Covariate name	Null model	Basic	Weather	Habitat	Land use Interactions	Land use+	Final model
Temporal	Intercept	✓	✓	✓	✓	✓	✓	✓
	Day number	✓	✓	✓	✓	✓	✓	✓
	Day number^2	✓	✓	✓	✓	✓	✓	✓
	Year		✓	✓	✓	✓	✓	✓
Climate	Temperature			✓				✓
	Precipitation			✓				✓
	Wind Speed			✓				
	Frost days			✓				✓
	Winter Precipitation			✓				
Habitat	Herb Species				✓			✓
	Tree Species				✓			✓
	Nitrogen				✓			
	pH				✓			
	Moisture				✓			
	Light				✓			✓
	Ellen. Temperature				✓			✓
	Habitat cluster 2	✓	✓	✓	✓	✓	✓	
	Habitat cluster 3	✓	✓	✓	✓	✓	✓	
Landscape	Arable land					✓	✓	✓
	Grassland					✓	✓	✓
	Forest					✓	✓	✓
	Water					✓	✓	✓
Interactions	Year × Day number		✓	✓	✓	✓	✓	✓
	Year × Day number^2		✓	✓	✓	✓	✓	✓
	Year × Agriculture						✓	✓
	Year × Forest						✓	✓
	Year × Water						✓	
	Year × Grassland						✓	✓
Variance	σ_{site}	✓	✓	✓	✓	✓	✓	✓
	v	✓	✓	✓	✓	✓	✓	✓

light, temperature and moisture, per location-year combination. Land use effects explored the fractions of agricultural area, forest, grass, and surface water in a radius of 200m around the plot location.

Parameter values are obtained by the use of Markov chain Monte Carlo (MCMC) methods by the aid of JAGS (Just Another Gibbs Sampler [50]) invoked through R [51] and the R2Jags package [52]. JAGS model scripts are given in S1 Code, while data are given in S1 and S2 Dataset. For each model, we ran 3 parallel chains each consisting of 24000 iterations (first 4000 discarded), and kept every 10th value as a way to reduce within chain autocorrelation. We used vague priors for all parameters, with uniform distributions for the residual and random effect variance components, and flat normal distributions (with very high variance) for all other parameters. Covariates in **X** were standardized prior to model fitting, with the exception of year (values 1–26), and land use variables (proportions within 0–1 range).

For all models, we computed the Deviance Information Criterion [53] (DIC) as well as the squared correlation coefficient (R^2) between observed and mean posterior estimates of biomass on the log scale. Results are given in Table 3. Parameter convergence was assessed by the

Table 3. Results for 7 models ranked by Deviance Information Criterion (DIC). For each model, the number of parameters, the Deviance Information Criterion, the effective number of parameters (pD), calculated R^2 and difference in DIC units between each model and the model with lowest ΔDIC. See Table 2 for covariates included in each model.

model	npar	Deviance	DIC	pD	R²	ΔDIC
Final	23	12082.48	12177.07	94.59	0.67	0.00
Weather	13	12178.84	12261.52	82.68	0.65	84.45
Land use+ Interactions	16	12336.22	12427.16	90.95	0.62	250.09
Habitat	15	12354.95	12445.93	90.98	0.62	268.86
Land use	12	12377.27	12453.23	75.97	0.61	276.16
Basic	8	12390.26	12465.08	74.82	0.61	288.00
Null	5	13230.65	13307.59	76.94	0.39	1130.52

potential scale reduction factor [54] (commonly $\hat{R}$), that measures the ratio of posterior distributions between independent MCM chains (in all models, all parameters attained values below 1.02). For all models, we confirmed that the posterior distribution of the trend coefficient did not confound any other variable by plotting the relevant posterior samples and computing pairwise correlation coefficients.

Our basic model included habitat cluster (3 levels), a quadratic effect for day number, an annual trend coefficient measuring the rate of biomass change, and the interactions between the annual trend coefficient and the day number variables. Next we developed 3 models each consisting of either weather variables (S1 Table), land use variables (S2 Table), or habitat variables. Because interactions between the annual rate of change and land use variables seemed plausible, a fourth model was developed to include these interactions (S3 Table). Finally, all significant variables were combined into our final model (Table 4), which included effects of an annual trend coefficient, season (linear and quadratic effect of day number), weather (temperature, precipitation, number of frost days), land use (cover of grassland and water, as well as interaction between grassland cover and trend), and habitat (number of herb and tree species as well as Ellenberg temperature).

Our estimate of decline is based on our basic model, from which we can derive seasonal estimates of daily biomass for any given year. The basic model includes only a temporal (annual and seasonal effects, as well as interactions) and a basic habitat cluster distinction (additive effects only) as well as a random trap location effect. We here report the annual trend coefficient, as well as a weighted estimate of decline that accounts for the within season differences in biomass decline. The weighted insect biomass decline was estimated by projecting the seasonal biomass (1-April to 30-October) for years 1989 and 2016 using coefficients our basic model, and then dividing the summed (over the season) biomass of 2016 by the summed biomass over 1989.

Using our final model, we assessed the relative contribution (i.e. net effect) of the explanatory variables to the observed decline, both combined and independently. To this aim we projected the seasonal daily biomass for the years 1989 and 2016 twice: first we kept covariates at their mean values during the early stages of the study period, and second we allowed covariate values to change according to the observed mean changes (see S2 and S3 Figs). Difference in the total biomass decline between these two projections are interpreted as the relative contribution of the explanatory variables to the decline. The marginal (i.e. independent) effects of each covariate were calculated by projecting biomass increase/decline as result of the observed temporal developments in each variable separately, and expressing it as percentual change.

Our data provide repetitions across years for only a subset of locations (n = 26 out of 63). As such, spatial variation in insect biomass may confound the estimated trend. To verify that

Table 4. Posterior parameter estimates of the final mixed effects model of daily insect biomass. For each included variable, the corresponding coefficient mean, standard deviation and 95% credible intervals are given. P-values were calculated empirically based on posterior distributions of coefficients.

Class	Variable	mean	sd	2.50%	97.50%	P	
Temporal	Intercept	2.450	0.233	1.983	2.891	0.000	***
	log(λ)	-0.080	0.007	-0.094	-0.067	0.000	***
	Day number	-0.100	0.028	-0.155	-0.045	0.001	***
	Day number^2	-0.447	0.029	-0.504	-0.392	0.000	***
Weather	Temperature	0.304	0.022	0.263	0.347	0.000	***
	Precipitation	-0.071	0.034	-0.143	-0.009	0.014	*
	Frost days	-0.021	0.024	-0.067	0.025	0.194	
Land use	Habitat Cluster 2	0.420	0.162	0.080	0.729	0.007	**
	Habitat Cluster 3	0.332	0.237	-0.133	0.806	0.078	.
	Arable land	-1.063	0.184	-1.420	-0.709	0.000	***
	Forest	-0.522	0.216	-0.947	-0.121	0.007	**
	Grassland	0.819	0.233	0.367	1.265	0.000	***
	Water	-0.327	0.170	-0.659	0.005	0.027	*
Habitat	Herb species	-0.054	0.045	-0.137	0.037	0.119	
	Tree Species	0.104	0.032	0.041	0.167	0.000	***
	Ell. Nitrogen	0.181	0.065	0.051	0.311	0.003	**
	Ell. Light	0.162	0.039	0.088	0.236	0.000	***
	Ell. Temperature	-0.071	0.031	-0.131	-0.011	0.010	**
Interactions	Year × Day number	-0.003	0.001	-0.006	-0.000	0.017	*
	Year × Day number^2	0.010	0.001	0.007	0.013	0.000	***
	Year × Arable land	0.047	0.008	0.031	0.064	0.000	***
	Year × Forest	0.035	0.010	0.016	0.055	0.000	***
	Year × Grassland	-0.059	0.014	-0.086	-0.033	0.000	***
Random effects	σ_{site}	0.334	0.037	0.270	0.412		
Residual variation	ν	0.870	0.009	0.852	0.889		

this is not the case, we fitted our basic model (but excluding the day number and year interaction to avoid overparameterization) to the subset of our data that includes only locations that were sampled in more than one year. Seasonal profiles of daily biomass values are depicted in S4 Fig. Finally, we reran our basic model for the two (of the three) habitat clusters (for which sufficient data existed; see Biomass Data) separately in order to compare the rate of decline between them (S5 Fig).

Results

Following corrections for seasonal variation and habitat cluster (basic model, see Materials and methods), the annual trend coefficient of our basic model was significantly negative (annual trend coefficient = −0.063, sd = 0.002, i.e. 6.1% annual decline). Based on this result, we estimate that a major (up to 81.6% [79.7–83.4%]) decline in mid-summer aerial insect biomass has taken place since 1989 (Fig 2A). However, biomass loss was more prominent in mid-summer as compared to the start and end of the season (Fig 3A), indicating that the highest losses occur when biomass is highest during the season (Fig 2B). As such, a seasonally weighted estimate (covering the period 1-April to 30-October; see methods) results in an overall 76.7% [74.8–78.5%] decline over a 27 year period. The pattern of decline is very similar across locations that were sampled more than once (Fig 4), suggesting that the estimated temporal decline based on the entire dataset is not confounded by the sampling procedure. Re-estimation of the

A

B
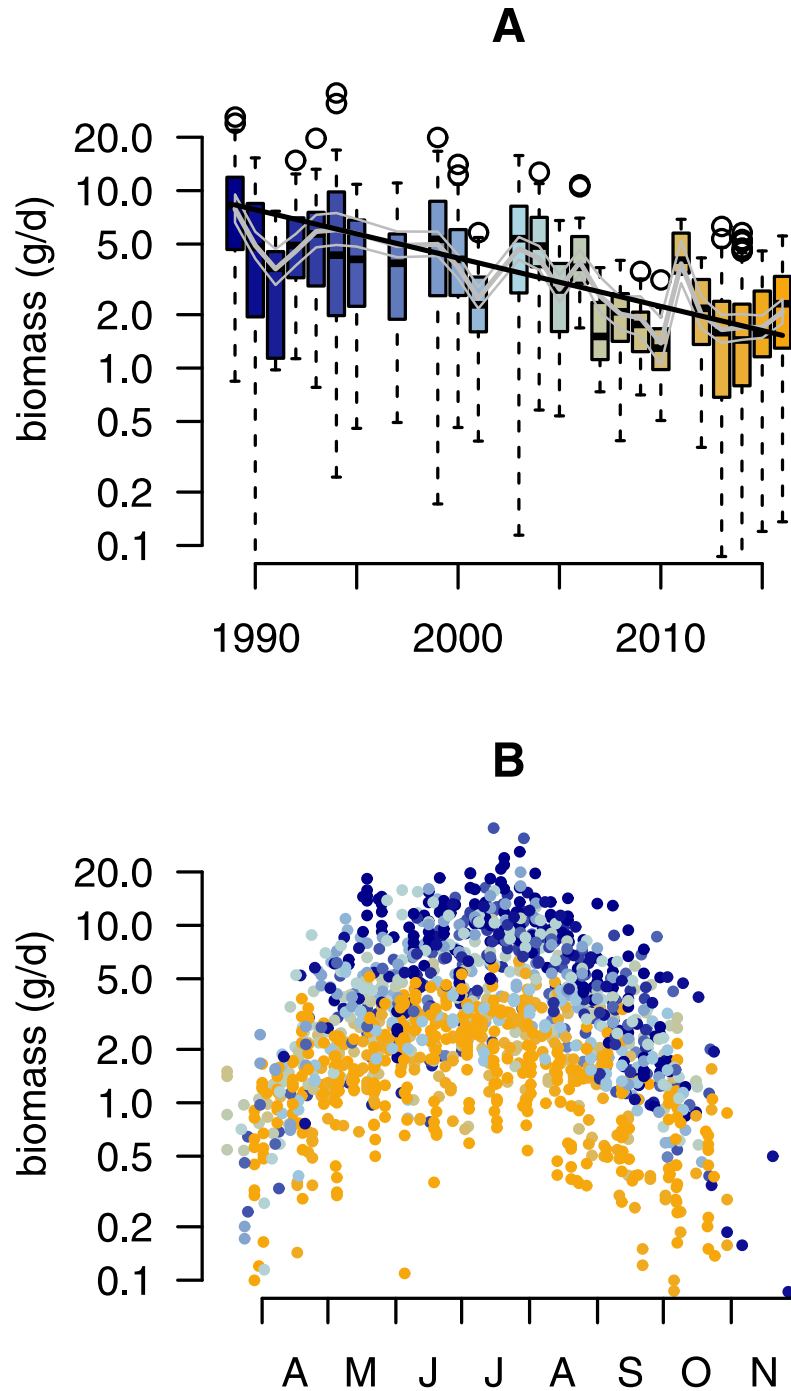
Fig 2. Temporal distribution of insect biomass. (A) Boxplots depict the distribution of insect biomass (gram per day) pooled over all traps and catches in each year (n = 1503). Based on our final model, the grey line depicts the fitted mean (+95% posterior credible intervals) taking into account weather, landscape and habitat effects. The black line depicts the mean estimated trend as estimated with our basic model. (B) Seasonal distribution of insect biomass showing that highest insect biomass catches in mid summer show most severe declines. Color gradient in both panels range from 1989 (blue) to 2016 (orange).

A

B
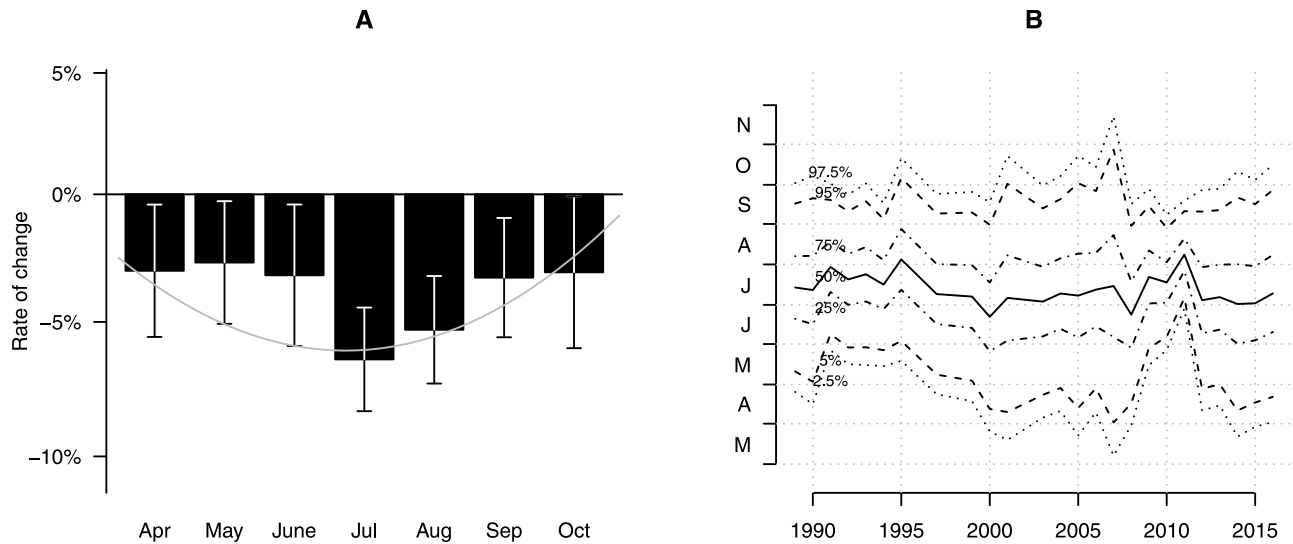
Fig 3. Seasonal decline and phenology. (A) Seasonal decline of mean daily insect biomass as estimated by independent month specific log-linear regressions (black bars), and our basic mixed effects model with interaction between annual rate of change and a quadratic trend for day number in season. (B), Seasonal phenology of insect biomass (seasonal quantiles of biomass at 5% intervals) across all locations revealing substantial annual variation in peak biomass (solid line) but no direction trend, suggesting no phenological changes have occurred with respect to temporal distribution of insect biomass.

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annual decline based on 26 locations that have been sampled in more than one year (S4 Fig), revealed a similar rate of decline (76.2%[73.9–78.3%]).

Insect biomass was positively related to temperature and negatively to precipitation (S1 Table). Including lagged effects of weather revealed no effect of either number of frost days, or winter precipitation, on the biomass in the next season (S1 Table). The overall model fit improved as compared to our basic model (R^2 = 65.4%, Table 3), explaining within and between year variation in insect biomass, but not the overall decline ($\log(\lambda)$ = −0.058, sd = 0.002). Over the course of the study period, some temporal changes occurred in the means of the weather variables (S2 Fig), most notably an increase by 0.5˚C in mean temperature and a decline 0.2 m/ sec in mean wind speed. Yet, these changes either do not have an effect on insect biomass (e.g. wind speed) either are expected to positively affected insect biomass (e.g. increased tempera- ture). Furthermore, a phenological shift with peak biomass earlier in the season could have resulted in lower biomass in the mid-season (Fig 3A), but this does not appear to be the case as none of the seasonal distribution quantiles in biomass showed any temporal trend (Fig 3B).

There was substantial variation in trapped insect biomass between habitat clusters (see Materials and methods), with nutrient-rich grasslands, margins and wasteland containing 43% more insect biomass than nutrient-poor heathland, sandy grassland, and dunes. Yet, the annual rate of decline was similar, suggesting that the decline is not specific to certain habitat types (S5 Fig). To further characterize trap locations, we used past (1989–1994) and present (2012–2015) aerial photographs and quantified land use cover within 200m around the trap locations. On average, cover of arable land decreased, coverage of forests increased, while grassland and surface water did not change much in extent over the last three decades (S3 Fig). Overall, adding land use variables alone did not lead to a substantial improvement of the model fit (R^2 = 61.3%, Table 3), nor did it affect the annual trend coefficient ($\log(\lambda)$ = −0.064, sd = 0.002). While presence of surface water appeared to significantly lower insect biomass, none of the other variables were significantly related to biomass. However, including interac- tions between the annual trend coefficient and land use variables increased the model fit
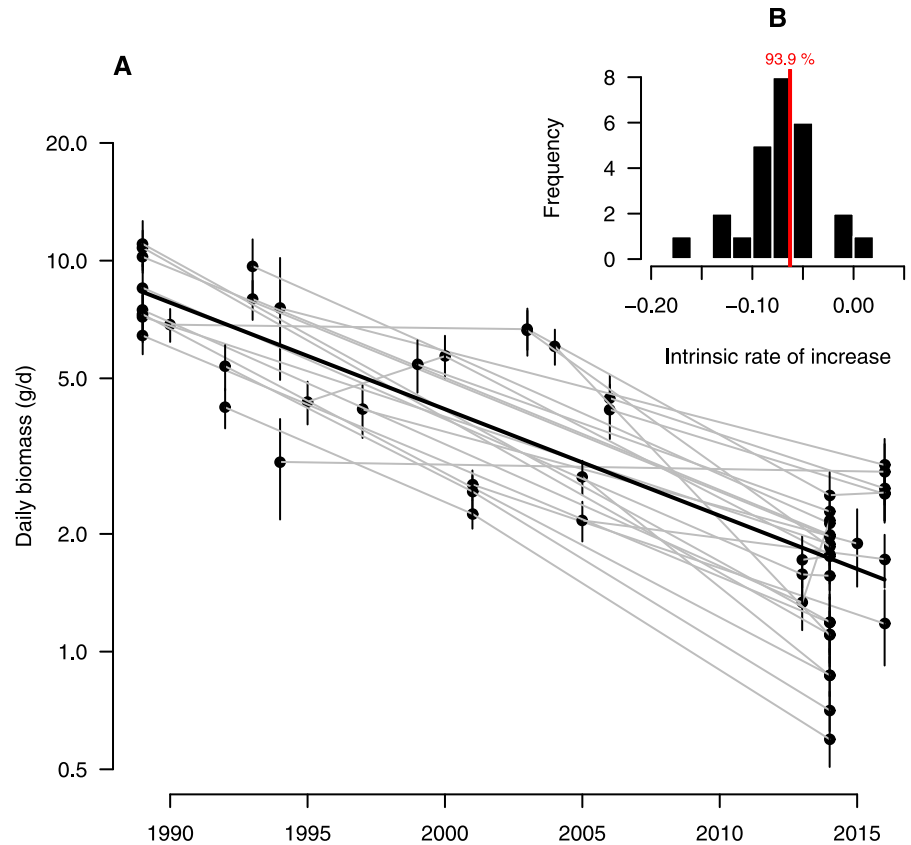
Fig 4. Temporal distribution of insect biomass at selected locations. (A) Daily biomass (mean ±1 se) across 26 locations sampled in multiple years (see S4 Fig for seasonal distributions). (B) Distribution of mean annual rate of decline as estimated based on plot specific log-linear models (annual trend coefficient = −0.053, sd = 0.002, i.e. 5.2% annual decline).

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slightly (Table 3), and revealed significant interactions for all variables except coverage of surface water (S2 Table). These interactions, which were retained in our final model (Table 4), revealed higher rates of decline where coverage of grassland was higher, while lower declines where forest and arable land coverage was higher.

We hypothesized that successional changes in plant community [55] or changes in environmental conditions [9, 18], could have affected the local insect biomass, and hence explain the decline. Plant species inventories that were carried out in the immediate vicinity of the traps and in the same season of trapping, revealed that species richness of trees, shrubs and herbs declined significantly over the course of the study period (S3 Fig). Including species richness in our basic model, i.e. number of tree species and log number of herb species, revealed significant positive and negative effects respectively on insect biomass, but did not affect the annual trend coefficient (S3 Table), explaining some variation between locations rather than the annual trend coefficient. Moreover, and contrary to expectation, trends in herb species richness were weakly negatively correlated with trends in insect biomass, when compared on per plot basis for plots sampled more than once. Ellenberg values of plant species provide a reliable indicator for the environmental conditions such as pH, nitrogen, and moisture [46, 47]. Around trap locations, mean indicators (across all locations) were stable over time, with changes in the order of less than 2% over the course of the study period. Adding these variables to our basic model revealed a significant positive effect of nitrogen and light, and a significant
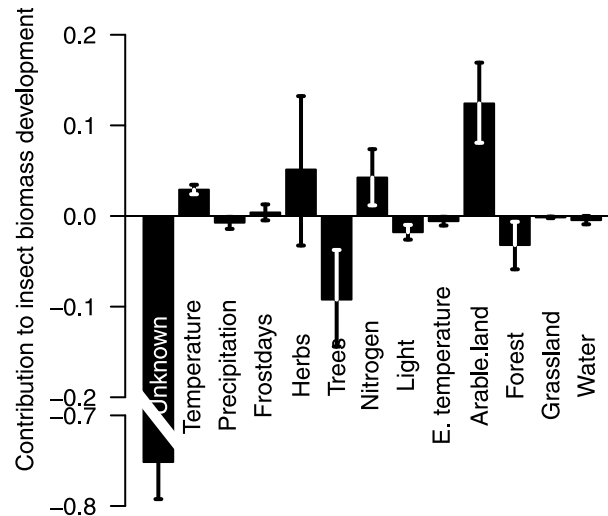
Fig 5. Marginal effects of temporal changes in considered covariates on insect biomass. Each bar represents the rate of change in total insect biomass, as the combined effect of the relevant coefficient (Table 4) and the temporal development of each covariate independently (S2 and S3 Figs).

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negative effect of Ellenberg temperature on insect biomass, explaining a major part of the variation between the habitat types. However, Ellenberg values did not affect the insect biomass trend coefficient ($\log(\lambda) = -0.059$, sd = 0.003, S3 Table) and only marginally improved the model fit ($R^2 = 61.9\%$, Table 3). All habitat variables were considered in our final model (Table 4), with the exception of of pH and moisture.

Our final model, based on including all significant variables from previous models, revealed a higher trend coefficient as compared to our basic model ($\log(\lambda) = -0.081$, sd = 0.006, Table 4), suggesting that temporal developments in the considered explanatory variables counteracted biomass decline to some degree, leading to an even more negative coefficient for the annual trend. The marginal net effect of changes in each covariate over time (see Analysis), showed a positive contribution to biomass growth rate of temporal developments in arable land, herb species richness, and Ellenberg Nitrogen, while negative effects of developments of tree species richness and forest coverage (Fig 5). For example, the negative effect of arable land on biomass (Table 4), in combination with a decrease in coverage of arable land (S3 Fig), have resulted in a net positive effect for biomass (Fig 5). Projections of our final model, while fixing the coefficient for the temporal annual trend $\log(\lambda)$ to zero, suggest insect biomass would have remained stable, or even increased by approximately 8% (mean rate = 1.075, 0.849–1.381) over the course of the study period.

Discussion

Our results document a dramatic decline in average airborne insect biomass of 76% (up to 82% in midsummer) in just 27 years for protected nature areas in Germany. This considerably exceeds the estimated decline of 58% in global abundance of wild vertebrates over a 42-year period to 2012 [56, 57]. Our results demonstrate that recently reported declines in several taxa such as butterflies [7, 25–27, 58], wild bees [8–14] and moths [15–18], are in parallel with a severe loss of total aerial insect biomass, suggesting that it is not only the vulnerable species, but the flying insect community as a whole, that has been decimated over the last few decades. The estimated decline is considerably more severe than the only comparable long term study on flying insect biomass elsewhere [28]. In that study, 12.2m high suction traps were deployed

at four locations in the UK over the time period 1973–2002, and showed a biomass decline at one of the four sites only. However, the sampling designs differ considerably between the two studies. Suction traps mainly target high-flying insects, and in that study the catches were largely comprised of flies belonging to the Bibionidae family. Contrary, malaise traps as used in the present study target insects flying close to the ground surface (up to 1 meter), with a much wider diversity of taxa. Future investigations should look into how biomass is distributed among insect species, and how species trends contribute to the biomass decline.

Although the present dataset spans a relatively large number of years (27) and sites (63), the number of repetitions (i.e. multiple years of seasonal distributions at the same locations) was lower (n = 26). We are however confident that our estimated rate of decline in total biomass resembles the true rate of decline, and is not an artifact of site selection. Firstly, our basic model (including an annual rate of decline) outperformed the null-model (without an annual rate of decline; ΔDIC = 822.62 units; Table 3), while at the same time, between-plot variation (i.s. σ_{site}) and residual variation (v) decreased by 44.3 and 9.7% respectively, after incorporating an annual rate of decline into the models. Secondly, using only data from sites at which malaise traps were operating in at least two years, we estimated a rate of decline similar to using the full dataset (Fig 4), with the pattern of decline being congruent across locations (S4 Fig). Taken together, there does not seem to be evidence that spatial variation (between sites) in this dataset forms a confounding factor to the estimated temporal trend, and conclude that our estimated biomass decline is representative for lowland protected areas in west Germany.

In light of previously suggested driving mechanisms, our analysis renders two of the prime suspects, i.e. landscape [9, 18, 20] and climate change [15, 18, 21, 37], as unlikely explanatory factors for this major decline in aerial insect biomass in the investigated protected areas. Habitat change was evaluated in terms of changes in plant species composition surrounding the standardized trap locations, and in plant species characteristics (Ellenberg values). Land use changes was evaluated in terms of proportional surface changes in aerial photographs, and not for example changes in management regimes. Given the major decline in insect biomass of about 80%, much stronger relationships would have been expected if changes in habitat and land use were the driving forces, even with the somewhat crude parameters that were at our disposal.

The decline in insect biomass, being evident throughout the growing season, and irrespective of habitat type or landscape configuration, suggests large-scale factors must be involved. While some temporal changes in climatic variables in our study area have taken place, these either were not of influence (e.g. wind speed), or changed in a manner that should have increased insect biomass (e.g temperature). However, we have not exhaustively analysed the full range of climatic variables that could potentially impact insect biomass. For example prolonged droughts, or lack of sunshine especially in low temperatures might have had an effect on insect biomass [59–62]. Agricultural intensification [17, 20] (e.g. pesticide usage, year-round tillage, increased use of fertilizers and frequency of agronomic measures) that we could not incorporate in our analyses, may form a plausible cause. The reserves in which the traps were placed are of limited size in this typical fragmented West-European landscape, and almost all locations (94%) are enclosed by agricultural fields. Part of the explanation could therefore be that the protected areas (serving as insect sources) are affected and drained by the agricultural fields in the broader surroundings (serving as sinks or even as ecological traps) [1, 63–65]. Increased agricultural intensification may have aggravated this reduction in insect abundance in the protected areas over the last few decades. Whatever the causal factors responsible for the decline, they have a far more devastating effect on total insect biomass than has been appreciated previously.

The widespread insect biomass decline is alarming, ever more so as all traps were placed in protected areas that are meant to preserve ecosystem functions and biodiversity. While the

gradual decline of rare insect species has been known for quite some time (e.g. specialized butterflies [9, 66]), our results illustrate an ongoing and rapid decline in total amount of airborne insects active in space and time. Agricultural intensification, including the disappearance of field margins and new crop protection methods has been associated with an overall decline of biodiversity in plants, insects, birds and other species in the current landscape [20, 27, 67]. The major and hitherto unrecognized loss of insect biomass that we report here for protected areas, adds a new dimension to this discussion, because it must have cascading effects across trophic levels and numerous other ecosystem effects. There is an urgent need to uncover the causes of this decline, its geographical extent, and to understand the ramifications of the decline for ecosystems and ecosystem services.

Supporting information

S1 Appendix. Malaise trap permissions.
(PDF)

S2 Appendix. Malaise traps.
(PDF)

S1 Code.
(PDF)

S1 Dataset.
(CSV)

S2 Dataset.
(CSV)

S1 Fig. Map of study area. Insect trap locations (yellow points) in Nordrhein-Westfalen (n = 57), Rheinland-Pfalz (n = 1) and Brandenburg (n = 5), as well as weather stations (crosses) used in the present analysis.
(TIFF)

S2 Fig. Temporal variation in weather variables. Annual means (A-C), daily means (D-F), and mean daily residual values (G-I) of temperature, precipitation and wind speed respectively. In all panels, black lines depict data while blue and red lines represent long term and seasonal fitted means of the variables, respectively.
(PDF)

S3 Fig. Land use and plant species richness changes. Mean land use in 1989–1994 (A) and 2012–2014 (B), based on aerial photograph analysis at 63 protected areas show a decrease of arable land and an increase in forested area over the past 25 years. (C) Changes in plants species richness for herbs (black) shrubs (red) and trees (blue). Annual means as well as mean trends are depicted in the corresponding colors. Linear trends are based on generalized linear mixed effects models with a Poisson error distribution and a random intercept effect for location. Note, zero values for tree and shrub species not depicted.
(PDF)

S4 Fig. Seasonal profiles of daily biomass across 26 locations. For each location, different colors represent different years, with time color-coded from green (1989) to red (2016). X-axis represents day number (January 1 = 0).
(PDF)

S5 Fig. Daily biomass of insects over time for two habitat clusters. Boxplots depict the distribution of insect biomass pooled over all traps and catches in each year at trap locations in nutrient-poor heathland, sandy grassland, and dunes (A), and in nutrient-rich grasslands, margins and wasteland (B). Grey lines depict the fitted mean (+95% posterior credible intervals), while the black lines the mean estimated trend. Estimated annual decline amounts to 7.5%(6.6–8.4) for habitat cluster 1, as compared to 5.2% (4.8–5.5) habitat cluster 2. Models fitted independently for each habitat location. Color gradient in all panels range from 1989 (blue) to 2016 (orange).
(PDF)

S1 Table. Posterior parameter estimates of the mixed effects model including weather variables. For each included variable, the corresponding coefficient posterior mean, standard deviation and 95% credible intervals are given. P-values are calculated empirically based on posterior distributions of coefficients.
(PDF)

S2 Table. Posterior parameter estimates of the mixed effects model including land use variables and interactions. For each included variable, the corresponding coefficient posterior mean, standard deviation and 95% credible intervals are given. P-values are calculated empirically based on posterior distributions of coefficients.
(PDF)

S3 Table. Posterior parameter estimates of the mixed effects model including habitat variables. For each included variable, the corresponding coefficient posterior mean, standard deviation and 95% credible intervals are given. P-values are calculated empirically based on posterior distributions of coefficients.
(PDF)

Acknowledgments

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Writing – review & editing: Caspar A. Hallmann, Martin Sorg, Eelke Jongejans, Henk Siepel, Heinz Schwan, Werner Stenmans, Andreas Müller, Hubert Sumser, Thomas Hörren, Dave Goulson, Hans de Kroon.

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Nonindigenous Species of the Pacific Northwest: An Overlooked Risk to Endangered Salmon?

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Nonindigenous species, which are associated with the decline of many threatened and endangered species, are a major threat to global diversity. This risk extends to salmonids, the most widespread threatened and endangered species in the Pacific Northwest. Pacific salmonids traverse large geographic areas that include freshwater, estuarine, and ocean habitats in which they encounter numerous nonnative species. For this article, we examined the extent to which introduced species are a risk to threatened and endangered salmon. We identified all documented nonindigenous species in the Pacific Northwest, including fish, invertebrates, birds, plants, and amphibians. Where data exist, we quantified the impact of nonindigenous species on threatened and endangered salmonids. The results indicate that the effect of nonindigenous species on salmon could equal or exceed that of four commonly addressed causes of adverse impacts—habitat alteration, harvest, hatcheries, and the hydrosystem; we suggest that managing nonindigenous species may be imperative for salmon recovery.

Keywords: nonindigenous species, predation impacts, Pacific Northwest salmonids, Columbia River, Endangered Species Act

Nonindigenous species (NIS) pose one of the dominant environmental threats to biological diversity (Vitousek et al. 1996, Simberloff et al. 2005) and are cited as a cause of endangerment for 48% of the species listed under the US Endangered Species Act (ESA) (Czech and Krausman 1997, Wilcove et al. 1998). In 2005, NIS cost the US economy in excess of $120 billion (Pimentel et al. 2005), and the occurrence and ranges of NIS are steadily increasing. Despite these high environmental and economic costs, little funding is devoted to tracking the distribution and spread of NIS (Crall et al. 2006, Lodge et al. 2006). Consequently, we do not know enough about NIS impacts on native species to make educated prevention and management decisions (Parker et al. 1999). This lack of information is especially of concern with regard to threatened or endangered species.

The introduction and establishment of nonindigenous fishes has contributed to the decline of native species worldwide (Lever 1996, Helfman 2007). The US Pacific Northwest (Washington, Oregon, and Idaho) is home to 119 plant and animal species that are federally listed as threatened or endangered, including Pacific salmon (NMFS 1998). Introduced species have been identified as a factor in the decline of many of these listings. During their life cycle, salmonids traverse large geographic areas spanning freshwater, estuarine, and ocean habitats where they encounter numerous nonnative species. These include a number of warmwater fish species that were introduced from eastern North America to provide recreational fishing opportunities. In the Columbia River system alone, juvenile Pacific salmon will encounter no fewer than eight documented nonindigenous predator and competitor fish species en route to the estuary. Many invertebrate and plant species that were also introduced into this system have documented impacts on native communities (Boersma et al. 2006). Despite the collective threat to native ecosystems, no study to date has examined the broad distributional patterns of NIS. Specifically, large-scale efforts to summarize the status of Pacific salmon have generally overlooked the distribution and impact of NIS in freshwater habitats (NRC 1996, Augerot 2005). When they have occurred, discussions of NIS have been specific to individual

fish species (e.g., Beamesderfer and Ward 1994, Fritts and Pearsons 2004). Because efforts to quantify the site-specific impacts of nonnative species have focused on single species interactions, no attempt has been made to understand the cumulative impact of these species on threatened or endangered salmonids or on their habitat.

To complicate matters, spatially explicit data describing the occurrence and distribution of terrestrial, aquatic, and marine NIS throughout the Northwest are not readily accessible to scientists, managers, or the general public (Crall et al. 2006). The US Geological Survey (USGS) maintains a national database of aquatic NIS, the University of Montana maintains a database of exotic plant species in the Northwest, and local reports document many incidental sightings of NIS. However, no comprehensive, spatially explicit database of terrestrial, aquatic, and marine NIS currently exists.

We have attempted to rectify this situation by assembling all known occurrence and distribution records for terrestrial and aquatic NIS into a comprehensive and spatially explicit database. We summarize these occurrences to describe the distributional pattern of NIS in this region. In a given watershed, we quantify the proportion of native and nonnative fish species and ask whether regions with higher numbers of NIS also have higher numbers of threatened and endangered species. The results of this simple correlation point to the need for mechanistic studies evaluating NIS impacts. We identify the potential mechanisms of impact, highlighting predation on salmonids as one example of the many consequences of species introductions. By synthesizing the results from individual site-specific predator studies, we demonstrate that notable predation impacts have been recorded. We emphasize the need to move beyond site-specific research and develop assessments of cumulative NIS impacts on salmon that can be compared with the commonly studied impacts of harvest, hatcheries, the hydrosystem, and habitat alteration—

often referred to as the all-H's. Finally, we quantify the amount of funding allocated for NIS studies compared with funds apportioned to the all-H's. Collectively, our efforts draw attention to the widespread distribution of NIS and their potential role in hastening the decline and impeding the future recovery of threatened and endangered salmon in the Pacific Northwest.

Nonindigenous species in the Pacific Northwest

Knowledge about the numbers and distribution of NIS in the Pacific Northwest is needed to assess the magnitude of their ecological impact across the region. As part of this analysis, it is necessary to document new introductions and delineate the spread of established nonnative species. To this end, we have created a spatially explicit database documenting the presence or absence of NIS to define the geographic locations of NIS within the states of Washington, Oregon, and Idaho (figure 1). We have incorporated data from local, regional, and national databases, as well as from published reports (table 1). We would have liked to have included estimates of abundance or biomass of nonnative taxa to associate with NIS presence or absence; however, these population demographic data are very scarce. This information will be required if we are to quantify the cumulative impacts of NIS. Our compilation effort identified numerous terms used to describe NIS, including "exotic," "invasive," "nonnative," and "alien." In this article we use the two most common—"nonindigenous" and "nonnative"—and employ them interchangeably.

This spatially explicit database represents a comprehensive synthesis of the information currently available regarding the distributions of NIS across the Pacific Northwest. Although our database incorporates all readily available information in this region, it is undoubtedly biased by the goals and motivations of the disparate sampling efforts, and thus should be presumed to represent minimum distributions

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Table 1. Database sources used to identify the spatial distribution of nonindigenous species in the Pacific Northwest.

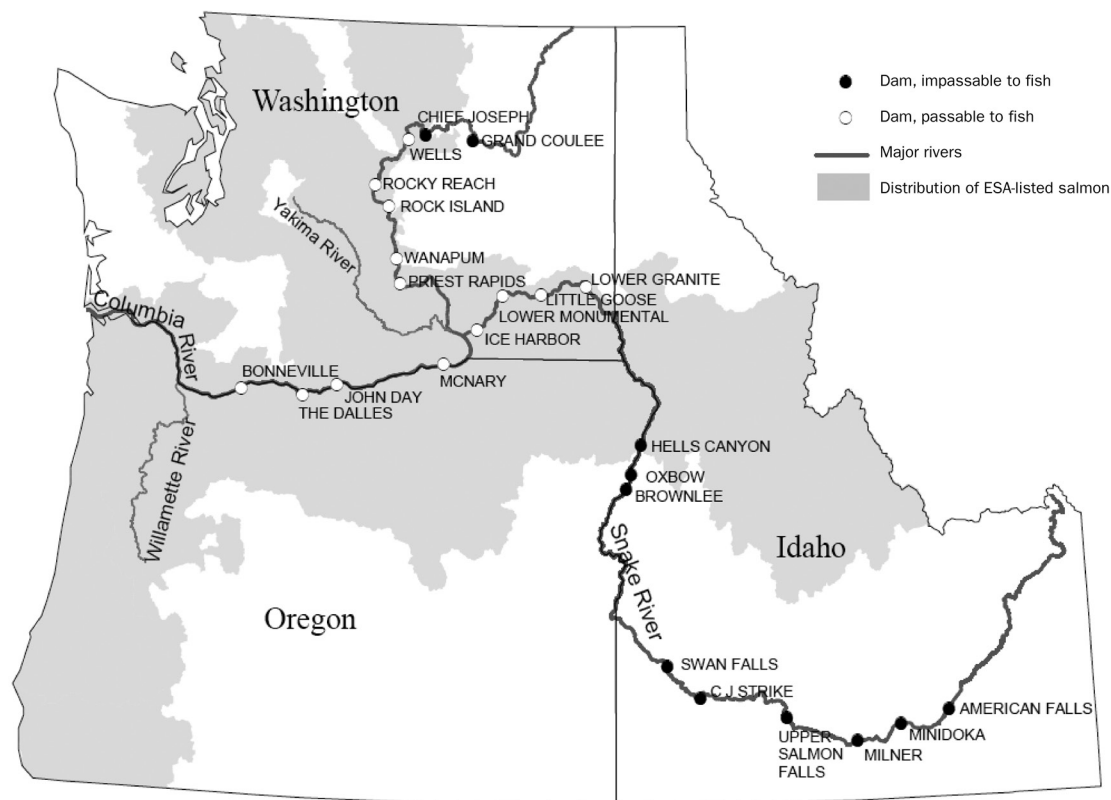| Taxonomic group	Data sources
Fishes	Daily K, Shrader T, Temple R, Hooton B. 1999. Introduced Fishes Management Strategies Public Review Draft 4-14-1999. Portland: Oregon Department of Fish and Wildlife.
	Northwest Power and Conservation Council. 2005. Subbasin Plan Amendments: Findings and Responses to Comments. (8 January 2009; www.subbasin.org/library/2005/2005-13.pdf)
	Temple R, Daily K, Shrader T, Hooton B. 1998. Predation issues: Introduced Fishes Draft Review Version. Portland: Oregon Department of Fish and Wildlife.
	US Forest Service, Boise National Forest Fish Species. (8 January 2009; www.fs.fed.us/r4/boise/recreation/fishing/fishing-old/documents/fish_species.shtml)
	Washington Department of Fish and Wildlife. 2005. Warmwater Fishes of Washington. Olympia: Washington Department of Fish and Wildlife. Report #FM93-9. (8 January 2009; http://wdfw.wa.gov/fish/warmwater/warmwater_2005.pdf)
	Wydoski RS, Whitney RR. 2003. Inland Fishes of Washington. Seattle: University of Washington Press.
Mudsnails	Montana State University. 2007. New Zealand Mudsnails in the Western USA. (8 January 2009; www.esg.montana.edu/aim/mollusca/nzms/)
Aquatic invertebrates, birds, mammals, reptiles, amphibians, fishes, and plants	US Geologic Survey. Nonindigenous Aquatic Species Database. (8 January 2009; http://nas.er.usgs.gov/)
	Northwest Habitat Institute. Corvallis, Oregon. (8 January 2009; www.nwhi.org)
Plants	Rice PM. INVADERS Database System Division of Biological Sciences. Missoula: University of Montana. (8 January 2009; http://invader.dbs.umt.edu)
	Washington State Aquatic Plant Survey, Washington Department of Ecology. (8 January 2009; www.ecy.wa.gov/programs/eap/lakes/aquaticplants/index.html#annualsurvey)

Figure 1. Major rivers, dams, and current distribution of threatened and endangered salmonids in the Pacific Northwest (gray shaded region). Historical distributions and distributions of salmonids not listed under the Endangered Species Act are not depicted.

in the region. Furthermore, the data we have compiled from other sources most likely represent a combination of established NIS as well as reported sightings for which establishment may be in question. Because we were most interested in organisms introduced from outside the Pacific Northwest, we defined NIS as species that were not natively found in Washington, Oregon, or Idaho. Thus, species native to one or more of these states were considered native. Furthermore, as species dispersal occurs over ecological rather than political units, our database was structured using watersheds defined by the USGS fourth field HUC (hydrologic unit code). These watersheds are roughly 1800-square-kilometer areas that represent hydrologically connected areas. Because our primary focus is on the interactions of NIS and salmon, we emphasize aquatic species, although the occurrences of species from other taxonomic groups—such as amphibians, birds, crustaceans, mammals, mollusks, plants, and reptiles—were systematically collected and included in the analysis. Surprisingly, data on the distribution of other major taxonomic groups in this region, such as insects and disease organisms, were unavailable.

Nonindigenous species are present in all regions of Washington, Oregon, and Idaho, with more than 400 NIS found in some watersheds (figure 2). Even those watersheds with the fewest NIS harbor nearly 100 species that were not present only
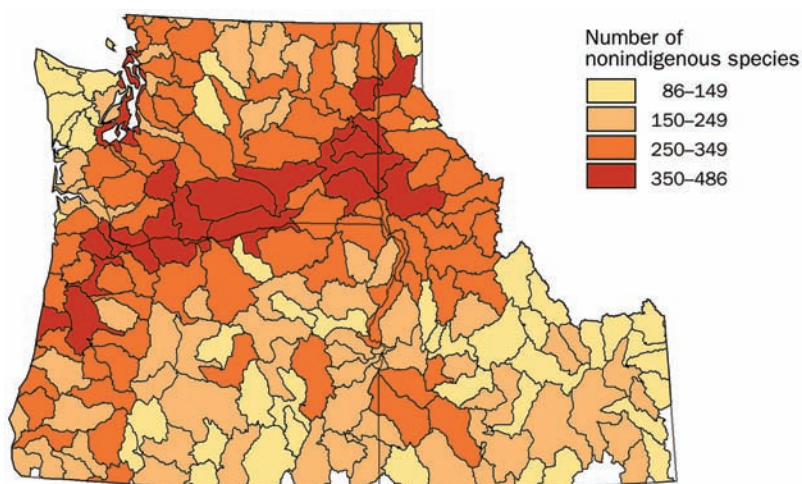

Figure 2. Number of nonindigenous species per fourth field HUC (hydrologic unit code) in Washington, Oregon, and Idaho. Taxonomic groups represented include plants, birds, fishes, amphibians, reptiles, mollusks, crustaceans, mammals, and other groups presented in figure 3.

two centuries ago. In total, more than 900 NIS have been documented within our study region, with the highest concentrations occurring along the Columbia River corridor and in areas with high human population density or intense agricultural activity, such as the Willamette River basin. The majority of NIS in the Pacific Northwest today are plants and fishes (figure 3). Common means of introduction include stocking for recreation (e.g., fish and birds); commerce (e.g., agricultural and landscaping plants, fish and bivalves used in aquaculture); biocontrol of nuisance species; escapes or releases, often of pets (e.g., fish, amphibians, reptiles); human transport through ballast water, biofouling, and fishing boats; and hitchhikers (e.g., fish, plants, crustaceans, mollusks, diseases) (Pimentel et al. 2005, Simberloff et al. 2005).
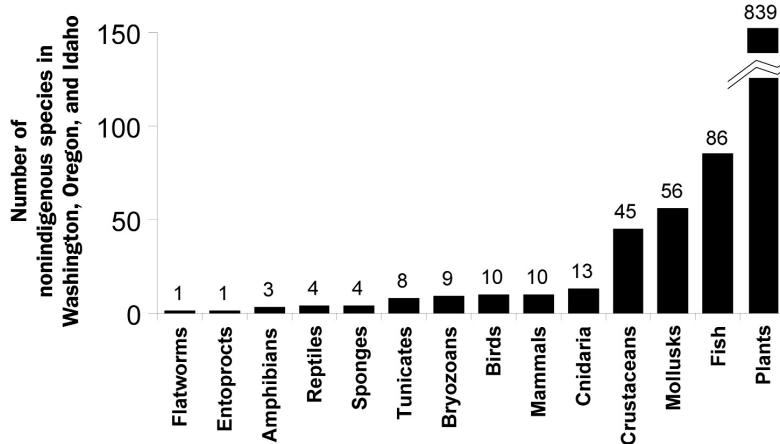
Figure 3. Number of nonindigenous species, by major taxonomic groups, in the Pacific Northwest states (Washington, Oregon, and Idaho).

Nonindigenous fishes and the decline of native fish species

The status of freshwater aquatic fauna is generally more dire than that of their terrestrial counterparts (Richter et al. 1997, Ricciardi and Rasmussen 1999, Rahel 2007). The presence of nonindigenous fishes poses one of the greatest threats to the persistence of healthy native fish populations (Lassuy 1995, Richter et al. 1997, Rahel 2002). Nationwide, introduced fish species have been cited as a factor leading to placement on federal threatened or endangered species lists in 70% of the fish listings (Lassuy 1995) and as a causal factor in 68% of the 40 North American fish extinctions in the last 100 years (Miller et al. 1989). In the western United States, one of every four stream fishes is nonnative, and the impact of nonnatives rivals that of habitat destruction (Schade and Bonar 2005). Using current data, the estimate of future extinction rates of freshwater fauna is approximately 4% per decade, a rate similar to that of tropical rainforest eco-

systems renowned for high rates of species losses (Ricciardi and Rasmussen 1999).

Nonindigenous fish species are ubiquitous throughout Washington, Oregon, and Idaho. The most heavily invaded watersheds have upward of 30 nonindigenous fishes. Similar observations have been made during recent stream surveys conducted in the western United States (Schade and Bonar 2005, Lomnicky et al. 2007). Nonnative aquatic vertebrates were present in more than 50% of surveyed stream reaches (Lomnicky et al. 2007), with even higher percentages in larger rivers, suggesting that a large portion of habitat occupied by native salmonids is shared with NIS. We observed the highest densities of nonnative fishes in southeastern Oregon and southern Idaho (figure 4). These high-density watersheds are located above Hells Canyon Dam and two other dams, which are impassable barriers to upstream migration of anadromous fish. Interestingly, recovery plans for threatened and endangered salmonids may require that salmonids be provided access to these currently blocked habitats where there are well-established populations of nonindigenous fishes such as channel catfish (*Ictalurus punctatus*), smallmouth bass (*Micropterus dolomieui*), yellow perch (*Perca flavescens*), and walleye (*Sander vitreus*).

Numbering around 60, nonindigenous fish species equal or outnumber native fishes (figure 5a), comprising 54%, 50%, and 60% of the fish species found in Washington, Oregon, and Idaho, respectively. Our estimates for the number of introduced species in these states are similar to or slightly higher than other published numbers, most likely because our database compilation effort is more comprehensive (Nico and Fuller
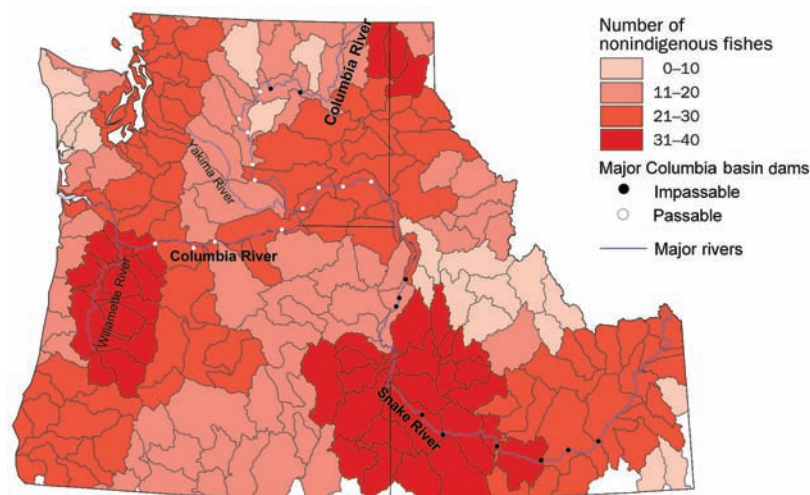

Figure 4. Number of nonindigenous fish species per fourth field HUC (hydrologic unit code) in Washington, Oregon, and Idaho. Dots represent major dams along the Columbia and Snake rivers. HUCs with less than 50% of their area in one or more of the three states were excluded from the spatial analyses and are not shaded (white).
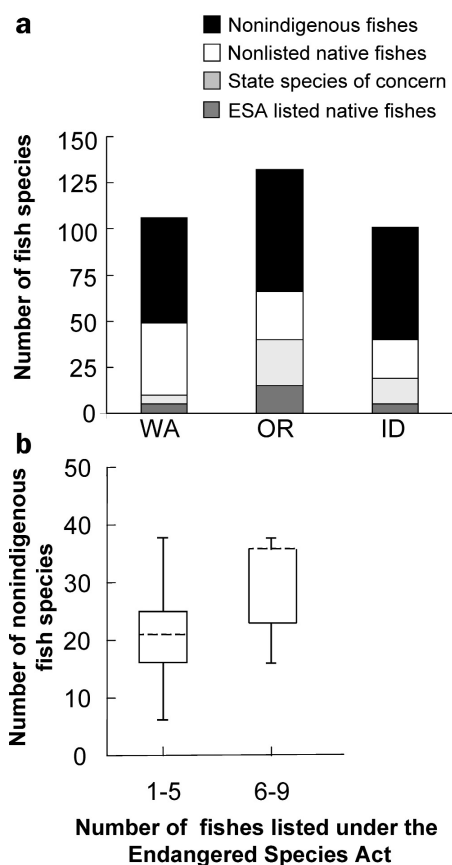
Figure 5. (a) Number of fish species in Washington, Oregon, and Idaho grouped by native or nonindigenous. Native fish species are categorized by status, including state species of concern (includes state endangered, threatened, and species of special concern that are not on the federal list), species listed under the Endangered Species Act (ESA), and species whose current status is satisfactory. (b) Number of nonindigenous fish species in watersheds (fourth field HUC [hydrologic unit code]) with few (1–5) and many (6–9) fishes listed under the ESA in the same HUC. Areas with greater numbers of threatened and endangered fishes have significantly more nonindigenous fish species (two-sample t-test, p = 0.001).

1999, Zaroban et al. 1999). Also, the data sources we have incorporated include records of established species as well as recorded observations of NIS that may or may not be established. Some of our data sources did not make this distinction, but among those that did, approximately 85% of the species listed were designated as "established." The abundance of nonindigenous fishes also reflects the homogenization of freshwater fauna reported across the country (Gido and Brown 1999, Rahel 2002). Of additional concern, 30% to 55% of the 40 to 60 native fishes found in each of the three states are federally listed as threatened or endangered, or are state species of special concern. Twenty-six fish species are federally listed as threatened or endangered in the three states: 17 Pacific salmon evolutionary significant units (*Oncorhynchus* spp.) (USFWS 2005), 3 species of chub (*Cyprinidae*), 3 species

of sucker (*Catostomidae*), the Foskett speckled dace (*Rhinichthys osculus*), the bull trout (*Salvelinus confluentus*), and the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). Of these 26 species listings, 71% cite NIS as a cause of endangerment in *Federal Register* notices. Furthermore, our data indicate higher numbers of threatened and endangered fishes in areas with greater diversity of nonindigenous fishes (figure 5b; two-sample t-test, $p < 0.001$). Although the co-occurrence of NIS fishes with threatened and endangered species cannot distinguish cause and effect from preference for similar habitats, the suggestion that nonnative fishes may play a role in the declining status of native fish species merits further evaluation (ISAB 2008).

Mechanisms of introduction and impact

Once they have been introduced and become established, NIS affect individual populations, communities, and ecosystem processes (Rosenzweig 2001, Simon and Townsend 2003). Across these scales, there are multiple mechanisms of impact, including predation, competition, hybridization, infection (disease and parasites), and habitat alteration (Mack et al. 2000, Simberloff et al. 2005). We researched the histories of several of the best known and most widely distributed nonindigenous fish, plant, and invertebrate taxa, many of which have documented or presumed negative impacts on Pacific salmon or on their habitats. The effects of NIS on salmon are not unique to the Pacific Northwest; throughout the world, NIS are a concern to the health of salmon populations, including salmon of eastern North America and Japan (NRC 2004, Helfman 2007, Han et al. 2008). Our case histories include examples of species that affect Pacific salmon ecosystems through three common mechanisms: predation, interactions with other species, and ecosystem modification. The histories identify when and how each species was introduced, synthesize knowledge of their impacts on Pacific salmonids and their habitats, and provide some insights into the rate at which spread has occurred.

American shad (*Alosa sapidissma*) colonized the Columbia River within years of being introduced in 1871 into the Sacramento River, California (Petersen et al. 2003). The spawning adult shad population in the Columbia River now numbers more than 5,000,000, the largest population of American shad in the world (Petersen et al. 2003). Although five times more shad than native salmon return yearly to the Columbia River, no studies have quantified the impacts of shad on salmon ecosystems. Only recently have scientists begun to examine the potential impacts of shad on Columbia River ecosystems, hypothesizing that planktivory by adult and juvenile shad reduces the availability of prey for juvenile salmonids, and further suggesting that the millions of juvenile shad migrating through the Columbia may fuel the growth and survival of other native and nonnative predators in the river that consume salmon (Petersen et al. 2003, Harvey and Karieva 2005, Haskell et al. 2006a). Results from studies to date indicate that juvenile shad prey heavily on zooplankton taxa, which are also a primary prey resource for

juvenile Chinook in the same habitats (Haskell et al. 2006a). Also, food-web models have been developed (Harvey and Karieva 2005) that indicate that juvenile shad may act as a prey subsidy to larger predators of salmonids.

As a result of extensive stocking efforts, brook trout (*Salvelinus fontinalis*) are now well established in streams throughout the Pacific Northwest. In 1913, the first operational brook trout hatchery opened in Washington. The ease of culturing brook trout, coupled with their high fecundity, hastened their spread, and by 1915 the hatchery had released more than one million fish (Karas 1997). The proliferation of brook trout has led to the decline of native bull trout and cutthroat trout through hybridization, displacement, competition, and predation (Gunckel et al. 2002, Dunham et al. 2004, Peterson et al. 2004). Although the potential impacts of brook trout on salmonids remain virtually unexplored, Levin and colleagues (2002) found that the presence of brook trout was associated with a 12% reduction in the survival of juvenile salmon in Snake River basin streams. The mechanism driving this difference in survival is unknown.

Predation is the most quantifiable impact of nonindigenous fishes on native species. Channel catfish, large and smallmouth bass, and walleye are four noteworthy predators in the Pacific Northwest (figure 6). Channel catfish require spawning water temperatures of 21 to 27 degrees Celsius. Consequently, only the Snake (Idaho), Yakima (Washington), Walla Walla (Washington), Tucannon (Washington), and Columbia rivers currently have naturally reproducing populations. In Columbia River reservoirs, large channel catfish (> 67 centimeters) consume thousands of juvenile salmon, which comprise 50% to 100% of their diets (Vigg et al. 1991). A single catfish eats an average of one juvenile salmon every three days in summer months (Vigg et al. 1991). To date, no studies have combined channel catfish population estimates with diet data to quantify the predatory impact of channel catfish on juvenile salmonids and other native species.

The construction of reservoirs associated with hydrosystem projects has facilitated the spread and establishment of many aquatic nonnative species, as well as the expansion of native species suited to these lotic environments (Harvey and Karieva 2005, Havel et al. 2005). This is certainly the case with smallmouth and largemouth bass (*M. dolomieui* and *Micropterus salmoides*), which are aggressive predators consuming virtually any prey smaller than the size of their gape, including fish, rats, mice, ducklings, frogs, snakes, and salamanders. The introduction of bass by private citizens began in the late 1800s, and since then bass have become well established throughout the region. In areas where freshwater bass have been introduced, predation by bass has contributed to the decline of some native fishes, frogs, and salamanders (Fuller et al. 1999). Although both smallmouth and largemouth bass prey on juvenile salmon, the impact is better documented for smallmouth bass, which consume 35% or more of juvenile salmon outmigrants in some regions (Fritts and Pearsons 2004). In addition, smallmouth bass have changed the size-based predation dynamics in some areas where they have largely displaced the native predator, northern pikeminnow (*Ptychocheilus oregonensis*; Fritts and Pearsons 2006). Unlike the case with pikeminnow, in which larger individuals have higher predation rates, smallmouth bass become piscivorous by two years of age, and their consumption of salmonids is highest for the smaller size classes (Fritts and Pearsons 2006).

Walleye were introduced much more recently than were most other nonnative species, but their means of introduction and spread are less documented. Since their introduction in the mid-1900s, walleye have colonized all reservoirs of the Columbia Basin Irrigation Project, most likely by migrating between reservoirs through irrigation pipes and canals (Lower Columbia Fish Recovery Board 2004). Walleye need warm water temperatures to spawn, and they have successfully established naturally reproducing populations, thus diminishing the incentive for continued stocking. Walleye are known to prey on juvenile Pacific salmon, consuming an estimated 250,000 to 2,000,000 smolts annually in the Columbia River (Rieman et al. 1991, Tinus and Beamesderfer 1994). The Columbia River basin is renowned for its walleye fishing, producing some of the largest individual fish on record.

In addition to NIS that compete with or prey on native taxa, our case histories include nonnative aquatic plant and invertebrate species that alter habitat and ecosystem functions. Nonindigenous aquatic invertebrates have been
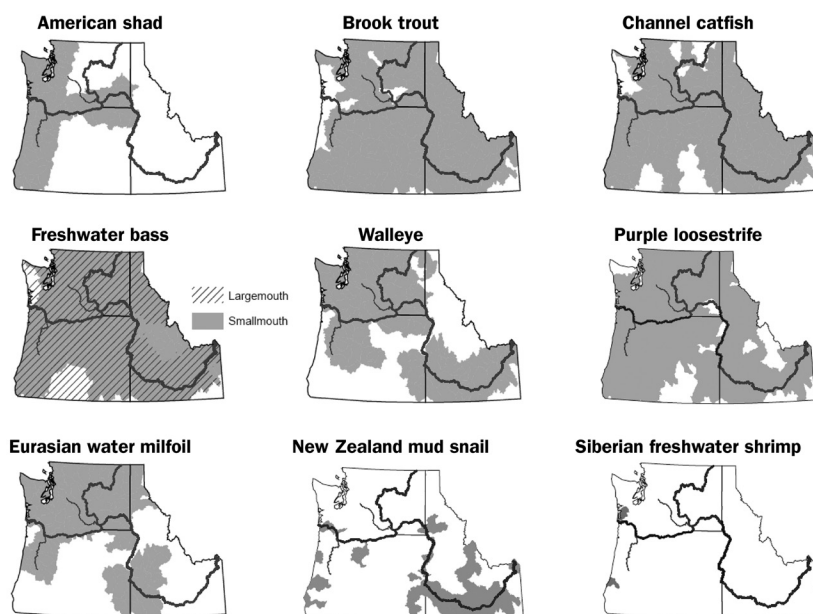

Figure 6. Distribution by fourth field HUC (hydrologic unit code) of nonindigenous fish with documented impacts on salmon, and aquatic plants and invertebrates with the potential to affect salmon streams.

implicated in the collapse of salmon populations elsewhere in the Pacific Northwest (Spencer et al. 1991). Despite the numerical abundance of nonnative plant and invertebrate taxa, the information needed to assess their impacts on aquatic and terrestrial ecosystems is rarely available, and thus the associated implications for habitats occupied by threatened and endangered salmonids are difficult to discern. Accordingly, these case histories are included here. Purple loosestrife (*Lythrum salicaria*) is a stereotypical invasive aquatic plant that was first found in the Pacific Northwest in 1929 (*www.ecy.wa.gov/ecyhome.html*). It grows rapidly, displacing native sedges and cattails (Blossey et al. 2001). Furthermore, this rapidly decomposing plant has the potential to produce a significant seasonal shift in local nutrient availability from a winter/spring flux to a fall flux. This shift may be detrimental to native fish species dependent on detrital food webs that peak in winter/spring (Blossey et al. 2001). Like purple loosestrife, Eurasian water milfoil (*Myriophyllum spicatum*) may also have been introduced by ballast soils from Europe in the 1800s (Aiken et al. 1979). This aquatic plant forms dense mats of vegetation that can depress dissolved oxygen concentrations at the sediment-water interface as they decompose, having significant effects on various aspects of aquatic ecosystem structure and function (Cronin et al. 2006, Unmuth et al. 2000).

Although their impacts have yet to be assessed, recent invertebrate introductions with the potential to influence salmon populations include the New Zealand mud snail (*Potamopyrgus antipodarum*) and Siberian freshwater shrimp (*Exopalaemon modestus*). The mechanism of the New Zealand mud snail's arrival in the mid-1980s is not known. Although small enough to fit on the tip of a match, these snails form dense colonies that can blanket streambeds. The mud snail can represent more than 95% of the invertebrate biomass in some areas, reaching densities of up to 500,000 individuals per square meter and exhibiting some of the highest reproduction rates observed for stream benthic invertebrates (Hall et al. 2003, 2006). Mud snail colonies have been reported to consume 75% of autochthonous gross primary production (Hall et al. 2003). They feed primarily on bottom-dwelling algae and detritus and can potentially outcompete other macroinvertebrates such as larval mayflies, stoneflies, and caddisflies (potential salmon prey) for food (Kerans et al. 2005). Mud snails have been identified in the stomachs of juvenile Chinook salmon sampled from the Columbia River estuary (Bersine et al. 2008). Whether these snails were intentionally ingested is not clear, but because of its thick shell and operculum, the snail is thought to be a poor nutritional source for salmon and other fish species, compared with insect larvae, fish, or other mollusks (Vinson and Baker 2008).

Unlike the mud snail, which clearly has the potential to affect native species, the potential impacts of the newly identified Siberian freshwater shrimp have not been studied. First detected in the lower Columbia River in 1995, the shrimp was very likely introduced by ship ballast water (Emmett et al.

2002). The prawn has rapidly extended its range and is already found in reservoirs in the lower Snake River (Haskell et al. 2006b). Although there have been no documented impacts to date, this nonnative shrimp may prey on native amphipods, such as *Corophium salmonis,* and directly compete with juvenile endangered salmon for important food resources (Emmett et al. 2002). Alternatively, it may provide a food source for native and nonnative resident fishes that also consume salmon (Haskell et al. 2006b).

We present only a few species histories here; however, each of the more than 700 NIS in the Pacific Northwest has its own unique story of introduction, establishment, and spread. Collectively, these stories demonstrate both how much and how little we know about NIS and their effects on native species and their ecosystems.

Predatory impacts of NIS: A literature review

Predation is the best-documented impact of NIS on Pacific Northwest salmon. Most of the information for the Columbia River basin has been obtained from small-scale studies performed on individual stream reaches or reservoirs, and restricted to an analysis of the impacts of encounters or interactions with a single NIS. Still, the limited numbers of empirical predation studies from the Columbia River basin outnumber those from other large river basins with Pacific salmon (Klamath River, California and Oregon; Sacramento River, California; Fraser River, British Columbia). Although focal studies indicate an enormous potential for NIS to affect salmon production and survival (Poe et al. 1991, Rieman et al. 1991, Vigg et al. 1991, Beamesderfer and Ward 1994, Baldwin et al. 2003), the necessary regional-scale, multispecies studies have not been undertaken.

By assembling all individual reports of predation on salmonids throughout the Columbia River basin, we can begin to ascertain the extent to which predation affects these threatened and endangered fishes. Accordingly, we reviewed all existing peer-reviewed and gray literature reporting the consumption of salmon by NIS for Pacific Northwest waterways. Our extensive literature search spanning published results and regional studies identified 27 studies documenting six nonindigenous fish species. Among these studies, diverse metrics were used to quantify consumption of juvenile salmon. Typical measures of predation reported were the percentage of a predator's diet that is composed of salmon and the total number of juvenile salmonids consumed by a predator population. Quantifying the impact of nonnatives requires an examination of both the density and biomass of nonnative taxa relative to native taxa. Only a few studies have attempted to examine predation in this way, reporting results as the percentage of an individual run of juvenile salmon devoured by a predator population. Studies have also reported differing levels of detail about the salmonids consumed. Some have reported prey consumed simply as "salmonids," whereas others specified species and, in some cases, whether the fishes were wild or hatchery produced. (Hatchery fishes are generally tagged or fin clipped before release.) Although

most of the studies that discriminated between hatchery and wild fishes found that predominantly hatchery fishes were consumed, predator populations built on high hatchery outputs may prey more heavily on wild salmon as a result of complex compensatory dynamics in these modified food webs (Lichatowich 1999, Fritts and Pearsons 2004). The circumstances surrounding each of these individual predation studies were seemingly as unique as the diversity of their reporting styles. All told, this diversity of approaches warrants a cautious use of the data. Our goal in assembling these records was to call attention to the cumulative impacts of NIS on salmonids.

Collectively, the data culled from our literature review indicate that the cumulative impact of NIS on salmonids is potentially substantial. Figure 7 summarizes results from the 27 studies that quantified predation by nonindigenous fishes on salmonids. In cases where ranges of values were presented (e.g., 200,000 to 400,000 salmon consumed per year), we plotted the median value for the range given (in this example, 300,000 salmon consumed per year). Of those studies reporting the number of juvenile salmon eaten by individual NIS predators, we found values that ranged from zero to 10.4 million (median value = 5.2 million), with many studies reporting hundreds of thousands of juveniles consumed by a single NIS predator species at a specific study site in the Columbia River basin. At locations in the Columbia River, smallmouth bass and walleye consumed between 18,000 to 2,000,000 and 170,000 to 300,000 juvenile salmonids per year, respectively. Similar predation rates were noted in all geographic areas (Columbia, Snake, and Yakima rivers, and Washington lakes and coast). Results from studies measuring the percentage of an outmigrating juvenile salmon run consumed by one predator species ranged between zero and 40% (figure 7). Studies of individual predator diets also yielded valuable information on the extent to which juvenile salmon were found in predator stomachs. For example, salmonids composed up to 100% of channel catfish diets in the Columbia and Yakima rivers,

and similar percentages were reported for smallmouth bass and walleye diets. Many of these studies quantified diets but did not subsequently determine the predator and prey population sizes needed to estimate an overall predation impact.

Considered in isolation, each of these studies provides minimal insight into cumulative predator impacts, and the divergent methods used by individual studies to record predation by NIS on salmonids make quantifying cumulative impacts virtually impossible. Yet, as salmon migrate up and down the Snake and Columbia rivers, they encounter 20 to 40 NIS, providing the opportunity for multiple direct effects (e.g., predation and competition) and indirect effects (e.g., food-web changes and habitat changes; figure 8). We recognize that co-occurrence of nonnative species does not necessarily correlate with higher impacts. Nonnative fishes may thrive in the Columbia for a number of reasons, including the creation of favorable reservoir habitat, the seasonal influx of juvenile salmon prey, or perhaps the declining numbers of salmon has provided new opportunities for colonization. Discerning cause and effect is difficult because basic information about the abundance, distribution, and ecological effects of most of these nonnatives is lacking. Studies examining predation impacts on salmonids have focused largely on native predators, namely, northern pikeminnow and avian predators such as Caspian terns and cormorants. We found 28 peer-reviewed papers, written since salmon were listed under the ESA in 1992, investigating six native predator species at 19 locations along the Snake and Columbia rivers. By comparison, we found only 22 peer-reviewed studies encompassing six NIS species from 17 sites in the Columbia River basin (figure 8a, 8b, lower panels). Furthermore, research efforts have focused mainly on reservoirs upstream of dams on the Columbia and Snake rivers. By synthesizing data on the spatial distribution and known impacts of NIS on salmonids throughout the Columbia River basin (figures 7, 8), we can begin a discussion of the overall effects of these NIS.
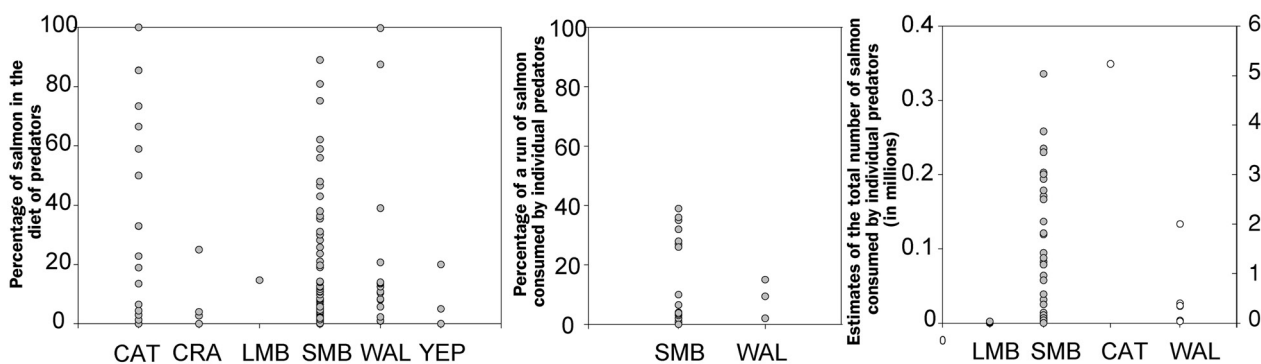

Figure 7. Results from literature review of 27 studies that document the percentage of salmon in the diet of predators (left panel), the percentage of a run of salmon consumed by individual predators (middle panel), and estimates of the total number of salmon consumed by individual predators in a given year (right panel). Species included are channel catfish (CAT), black and white crappie (CRA), large and smallmouth bass (LMB and SMB), walleye (WAL) and yellow perch (YEP). For the right panel, data for LMB and SMB (gray filled symbols) correspond to the left axis, and data for CAT and WAL (open symbols) correspond to the right axis.

Aliens in our midst: Are NIS ignored?

Throughout the Pacific Northwest, the causes of salmon population declines have been dominated by a discussion of the impact of the all-H's—hydrosystem, hatchery, harvest, and habitat. This all-H-centric view has largely ignored the impacts of key NIS in Pacific Northwest watersheds, which may rival the detrimental effects of the all-H's (Ruckelshaus et al. 2002). For example, on a per-run basis, the mortality attributed to NIS predation may be similar to that associated with juvenile passage through each of the eight dams on the Columbia and Snake rivers, estimated at approximately 5% to 15% per dam (Muir et al. 2001).

Similarly, predation by nonnative fishes on outmigrating smolts is roughly equivalent to the productivity declines attributed to habitat loss and degradation (Beechie et al. 1994). Furthermore, although it is difficult to make direct comparisons between adult and juvenile mortality with respect to population impacts, predation rates on juvenile outmigrants are also similar in magnitude to harvest-related mortality rates on adults (3% to 84%; McClure et al. 2003).

Despite clear evidence of the impact of NIS, a consideration of their role still falls outside all-H thinking. To illustrate this point and to quantify the level of funding directed to studies of nonnative species, we analyzed the $385 million that the Bonneville Power Administration (BPA) Fish and Wildlife program has allocated to research, restoration and enhancement projects from 2007 to 2009 (Eric Schrepel, Northwest Power and Conservation Council [NWPCC], Portland, Oregon, personal communication, March 2008). BPA is required by the Northwest Power Act of 1980 to mitigate the adverse environmental effects imposed by its 31 federal hydropower dams in the Columbia River basin. To do so, BPA awards competitive funding to third-party agencies (e.g., universities, tribal groups, and state agencies) that conduct research and manage natural resources associated with the Columbia River basin. Given their broad geographic and ecological scope, and the relative amount of funding in dollar terms, the projects funded by BPA can logically be presumed to reflect the priorities of scientific inquiry among other funding agencies in this region.

Assisted by the agencies' staff, we scrutinized the NWPCC database of funded projects for the years 2007–2009. First, we identified funded projects with a nonnative species component using key words such as pikeminnow, squawfish, noxious, warm water, nonindigenous, nonnative, exotic, bass, eradication, weed, and control. Next, through careful examination of flagged projects, we classified project funding in accordance with the purpose of the project: research, control, or enhancement of NIS. We evaluated only projects with descriptions available online at the Columbia Basin Fish and Wildlife Authority Web site (*www.cbfwa.org/ funding_main.cfm*). Funding for mixed com-
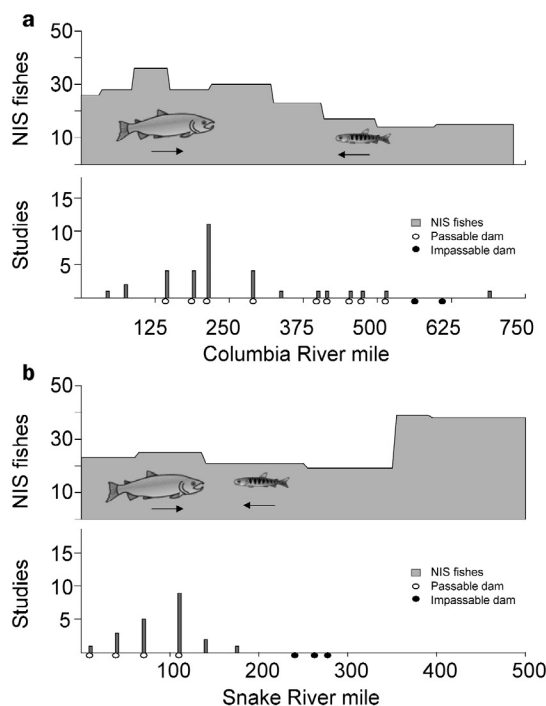

Figure 8. Number of nonindigenous fishes encountered by juvenile and adult salmonids as they migrate down and up the Columbia (a) and Snake (b) rivers. The Columbia River mile zero is located at the Columbia estuary, and the Snake River mile zero is located where the Snake merges with the Columbia River (river mile 325). The lower panel of each graph documents the number of published studies on nonindigenous species and the locations where these studies were conducted. Note that many of these studies were conducted near hydroelectric dams, which are identified by open (passable for salmonids) and closed (impassable for salmonids) circles.
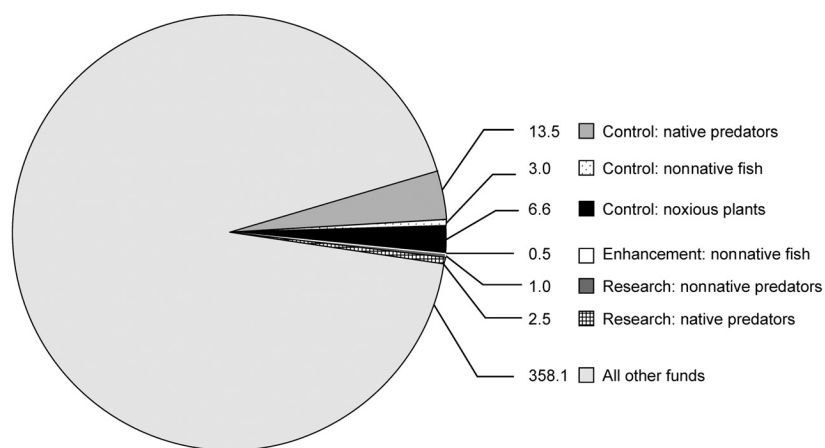

Figure 9. Funding (in millions of dollars) allocated to research and control of nonindigenous species in the Columbia River basin (2007–2009) by Bonneville Power Administration. Projects are categorized in accordance with their purpose: research on native or nonnative species, control (of noxious plants, nonnative fish, and native predators), enhancement of nonnative fish, and all other projects.

ponent projects was classified in an all-or-nothing manner such that funding for projects containing any research component was defined as research, whereas all other funding was allocated in accordance with the predominant project goal. Thus, the results reported here are biased toward funds allocated to nonnative research. Results of our survey indicate that of the $385 million distributed by BPA over the three-year study period, only approximately 0.3% was directed in whole or in part toward research on the impacts of NIS (figure 9), and slightly less than 1% of funds were allocated to efforts to control nonindigenous fish species. A greater proportion of funding (approximately $20 million, 5.2%) was spent on projects dedicated to the control and removal of noxious weeds and important native predators (e.g., pikeminnow and avian predators such as terns and cormorants). Although native to the region, these predator species have expanded their distribution and increased in abundance as a result of habitat modifications along the Columbia and Snake rivers. During this same period, $560,000 was spent on enhancement projects designed to introduce or maintain populations of selected nonnative species. Specific information about the continued stocking of nonnative fishes in the Pacific Northwest is available on state management Web sites. Management agencies are becoming more cautious about introducing and stocking nonindigenous fishes, yet the continued stocking of some nonindigenous fish species reflects the high value attached to sport fisheries in this region.

Considering the percentage of funds allocated to NIS research and the results of our review of impacts, the level of attention given to NIS seems disproportionately small, given the magnitude of the potential threat that NIS pose to native communities. For wide-ranging migratory species, quantifying the impacts of countless NIS that occur over hundreds of miles is a daunting challenge. Thus, the scale of the NIS problem far exceeds the scale of most management and research efforts (ISAB 2008). To date, efforts to examine the role of NIS have been largely limited to site-specific studies of individual species.

Future opportunities for understanding and managing NIS already exist within ongoing research and management programs. The value of site-specific studies grows as results of individual studies are integrated across spatial and temporal scales relevant to the salmon life cycle. For example, as a cohort of juvenile salmon travel from their natal habitats to the ocean, what proportion of those individuals is lost to predation by nonnative species? Because many of the major NIS predators are popular game fishes managed by state agencies, the predator biomass data needed to quantify predation rates on salmonids are quite likely available. Additionally, native predator programs exemplify how the region might develop similar programs to mitigate the damage imposed by NIS and improve the chances of recovery for native species at risk. Only with a broad examination of NIS ecology and impacts by both existing and new research programs can we begin to answer questions that are key to evaluating the cumulative impact of NIS on salmonids.

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