-3, into which the fluorescent proteins enhanced green fluorescent protein (EGFP) or mCherry were introduced to distinguish between the two cell lines. These cells were sparsely cultured to allow the formation of independent colonies. When their colony edges came into contact with one another, their boundaries were examined. Cells expressing identical nectin types did not intermingle at the border, whereas those expressing nectin-1 and -3 mutually invaded the counter colony, resulting in the formation of a mosaic pattern (Fig. 4, A to E, and fig. S6). We also performed time-lapse video microscopy using a coculture of MDCK cells expressing nectin-1 or -3 (N1- and N3-MDCK cells). In the supporting movie (movie S1 and Fig. 4E), one N1-MDCK cell (arrowhead) initially adhered to one of a pair of N3-MDCK cells (asterisks); subsequently, the former cell invaded the space between the two N3-MDCK cells. As a result, N1- and N3-MDCK cells were rearranged into a mosaic pattern. Similar behavior of cells was repeatedly observed in multiple experiments.

Thus, we propose that the heterophilic interactions between nectin-1 and -3 are critical for establishing the checkerboard-like pattern of hair cells and supporting cells. The molecular interaction between nectin-1 and -3 is the strongest of all possible combinations of the three nectins, which is likely to be responsible for the checkerboard-like assembly of these cells (Fig. 4F), as predicted by the mathematical model (8). The loss of nectin-3 removed such biased cell-cell adhesion, leading to cell rearrangement, including attachments between hair cells (Fig. 2D), as explained by the differential adhesiveness hypothesis (18). Nectin-1 KO mice displayed milder phenotypes. In these mice, the relatively strong interaction between nectin-3 and -2 probably retained the adhesion between hair cells and supporting cells; on the other hand, the adhesion between supporting cells should have been enhanced as a result of the redistribution of nectin-3 to these sites. These combinatory situations probably suppressed adhesion between hair cells (Fig. 4F). In nectin-2 KO mice, the heterophilic interactions between nectin-1 and -3 persisted; this explains the absence of a phenotype in these mice. In the absence of nectins, the cell junctions were not disrupted. This is most likely due to the coexpression of classic cadherins in the auditory epithelia. Hair cells and supporting cells are thought to be segregated through the process of lateral inhibition mediated by Notch-Delta signaling (4, 19), and such processes themselves might contribute to the spatial separation of these cells (20–22). However, genetic inactivation of Notch signaling does not impair the checkerboard-like pattern, although it does result in an increase in the number of hair cells (4). This suggests that lateral inhibition is insufficient to create the checkerboard-like cellular pattern, stressing the importance of nectins in this patterning process. It is of note that heterophilic interactions between Hibris and Roughest, other members of the immunoglobulin superfamily, also contribute to the cell arrangement in the *Drosophila* eye (23, 24), suggesting that similar mechanisms are conserved for cellular patterning across species.

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Impacts of Fishing Low–Trophic Level Species on Marine Ecosystems

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Low–trophic level species account for more than 30% of global fisheries production and contribute substantially to global food security. We used a range of ecosystem models to explore the effects of fishing low–trophic level species on marine ecosystems, including marine mammals and seabirds, and on other commercially important species. In five well-studied ecosystems, we found that fishing these species at conventional maximum sustainable yield (MSY) levels can have large impacts on other parts of the ecosystem, particularly when they constitute a high proportion of the biomass in the ecosystem or are highly connected in the food web. Halving exploitation rates would result in much lower impacts on marine ecosystems while still achieving 80% of MSY.

Concerns about the trophic impact of harvesting marine species were recognized more than three decades ago (1). Despite recent successes in reducing exploitation rates in some marine ecosystems (2), concerns remain over the effects of fishing on the structure and function of marine ecosystems (3, 4).

Low–trophic level (LTL) species in marine ecosystems comprise species that are generally plankton feeders for the larger part of their life cycle. They are often present in high abundance and tend to form dense schools or aggregations. They include small pelagic "forage" fish such as anchovy, sardine, herring, mackerel, and capelin but also invertebrate species such as krill. Humans harvest across the trophic levels in marine food webs, and landings of LTL species have been increasing generally in proportion with global catches (5). Forage fish account for over 30% of global fish landings, most of which is

now used for fishmeal production as feed for livestock industries and aquaculture rather than being consumed directly (*6*). However, LTL species also contribute directly to food security in many developing countries, and between 10 and 20% of global landings are consumed directly by humans (*7*). One species alone, Peruvian anchovy, contributes up to 50% of global landings used for fishmeal production. Driven by global markets for fertilizer, animal feed, and increases in the production of seafood from aquaculture, demand for fishmeal continues to increase (*8*).

LTL species play an important role in marine food webs because they are the principal means of transferring production from plankton to larger predatory fish and to marine mammals and sea-

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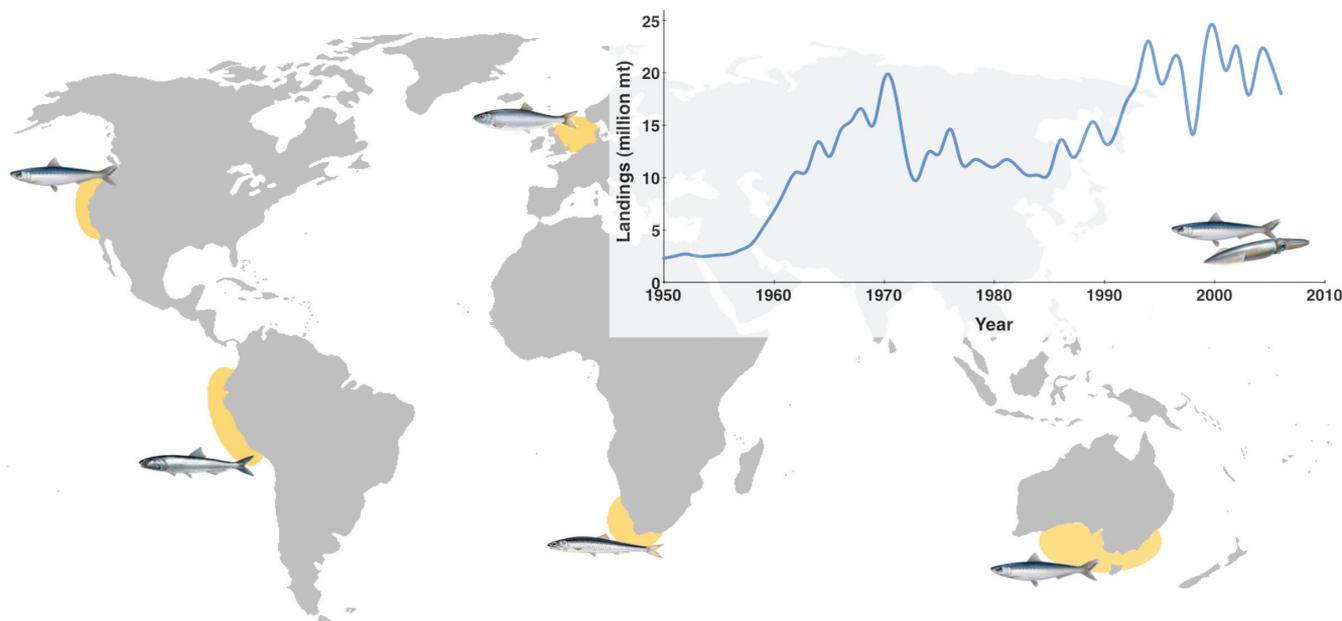*To whom correspondence should be addressed. E-mail: tony.d.smith@csiro.edu

birds. Several studies have raised concerns about the impacts on seabirds of local depletion of forage fish [anchovy in Perú (*9*), sand eels in the North Sea (*10*), and anchovy and sardines in South Africa (*11*)]. Similar concerns have been raised about the prospects of a large increase in catch of krill in the Southern Ocean and its potential impact on recovery of depleted marine mammals such as whales (*12*). Of particular concern are "wasp waist" systems, where a large part of the plankton production is funnelled through a small number of LTL species to higher trophic levels (*13*, *14*).

Although studies in individual ecosystems have raised concerns about the ecological effects of fishing LTL species, there has been no systematic attempt to examine and summarize what these broader effects might be or under what circumstances various effects might be expected to arise. In this study, we used ecosystem models in five well-studied regions to examine systemic effects of fishing LTL species. The regions include three eastern boundary current ecosystems—the northern Humboldt, the southern Benguela, and the California current—and two systems less dominated by upwelling, including the North Sea and the southeast Australian shelf and continental slope (Fig. 1). To avoid conclusions being dominated by structural assumptions in particular types of model, we used three different ecosystem models to explore the responses: Ecopath with EcoSim (EwE) (*15*, *16*), OSMOSE (*17*, *18*), and Atlantis (*19*, *20*). For each ecosystem and model, we selected up to five LTL species or groups and subjected them one by one to a range of fishing pressures, resulting in depletion levels

relative to unfished biomass from zero (no fishing) to 100% (extirpated). The LTL species selected included some that are currently fished (such as anchovy) and others that are not currently exploited in those ecosystems (such as krill and mesopelagic fishes). We did not include harvested shellfish such as scallops and prawns, notwithstanding their commercial importance (*21*), because most of the models did not resolve these species well. Impacts on other ecological groups in the ecosystem were measured relative to biomass levels of those groups produced by simulations in which the focal LTL species was unfished, and all other groups were fished at current levels. Details of the ecosystems, models, groups, and experiments are provided in (*22*).

We found widespread impacts of harvesting LTL species across the ecosystems and LTL species selected (Fig. 2). The percent of ecological groups exhibiting effects greater than 40% increased with the level of depletion of the LTL species, but the extent of impact also varied across LTL species. Impacts on other ecological groups were both positive and negative (fig. S1), ranging up to very severe impacts for some groups (>60% change in biomass) even at relatively low levels of depletion (25% below unfished levels—that is, biomass reduced to 75% of unfished levels) of the LTL species. Negative impacts (reductions in abundance) tended to predominate for marine mammals and seabirds, although the majority of impacts on such groups were small. Some commercial species could also be negatively affected, although again impacts on most commercial species were small. Results

Fig. 1. Global map showing location of study ecosystems. From left to right are the California current, northern Humboldt, North Sea, southern Benguela, and southeast Australia. Graph shows trend in landings of forage species from 1950 to 2009. [Source: Sea Around Us Project, www.seaaroundus.org/global/1/3.aspx. Images of forage fish are copyright Casson Trenor, 2010, at www.sustainablesushi.net]

were generally robust to the three types of model used in the analysis (fig. S2).

The variation in impact of harvesting different LTL species has potentially important management implications; large impacts may require a change in harvest levels, whereas LTL species with small impacts could be harvested at conventional single-species levels. In each ecosystem, harvesting several of the LTL species was found to have high impacts, although the species with high impacts were not always consistent across ecosystems (Fig. 2). For example, in the northern Humboldt ecosystem, harvesting anchovy had high impacts, and harvesting sardine had low impacts, but in the southern Benguela ecosystem, harvesting sardines had the larger impact, whereas the impacts of fishing both species were low in the southeast Australian and California current ecosystems. Impacts of harvesting mesopelagic fishes (a group not currently targeted in any of these ecosystems or generally in global fisheries) were consistently high across ecosystems, and impacts of harvesting krill (large zooplankton), also not currently exploited in these ecosystems, also tended to be medium to high. Fishing sand eels had the highest impact in the North Sea.

To explain this range of impacts across LTL species, we looked for more generic properties of these groups (other than taxonomy) that might explain and predict the variation. Three potential predictors were the relative abundance of the group in the ecosystem (for example, Peruvian anchovy accounts for up to 35% of the consumer biomass in the northern Humboldt ecosystem), the trophic level of the group, and the connectivity of the group in the food web. Trophic level was not a good predictor of impact, but the other two factors appear to be important. Abundant groups have consistently large impacts, whereas smaller groups can have either small or large impacts (Fig. 3A). There appears to be a threshold effect for connectance (the proportion of total trophic connections in the food web for each LTL species), with species that have a connectance value greater than ~0.04 having larger impacts (Fig. 3B). However, factors other than total connectance are likely to be important, including the presence of groups with trophic niches similar to those of the exploited species that can dampen the ecosystem effects of depleting the targeted species.

There are important tradeoffs to examine in considering the wider implications of these results for exploitation of LTL species. In particular, impacts on other parts of the ecosystem will be smaller at lower exploitation rates, but yields also will be lower (Fig. 4). There is a tension here between achieving broader goals of protecting and maintaining biodiversity (including ecosystem structure and function) and global food security. LTL species support the latter both through direct human consumption and through providing feed for livestock and aquaculture production. Considerable reductions in impact can be achieved by moving from exploitation at MSY levels (achieved at close to 60% depletion levels) to a target of 75% of unexploited biomass (25% depletion) for an LTL species, as shown in Fig. 4. The cost of such a change would be slightly less than 20% of long-term yield. This target could be achieved at significantly lower exploitation rates (mostly less than half MSY rates) (fig. S3), which would imply much lower fishing effort and may be closer to long-term economic optimum levels. There could also be some benefit of a reduction in harvest rate of LTL species to yields for other

Fig. 2. Effects of level of depletion of LTL species on the proportion of other trophic groups whose biomass varied by more than 40% relative to their level where the LTL species was not fished. Results are shown for a variety of LTL species fished in each modeled ecosystem.
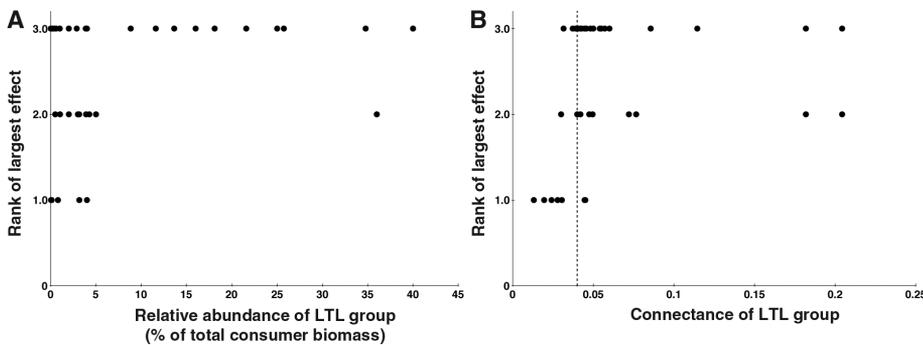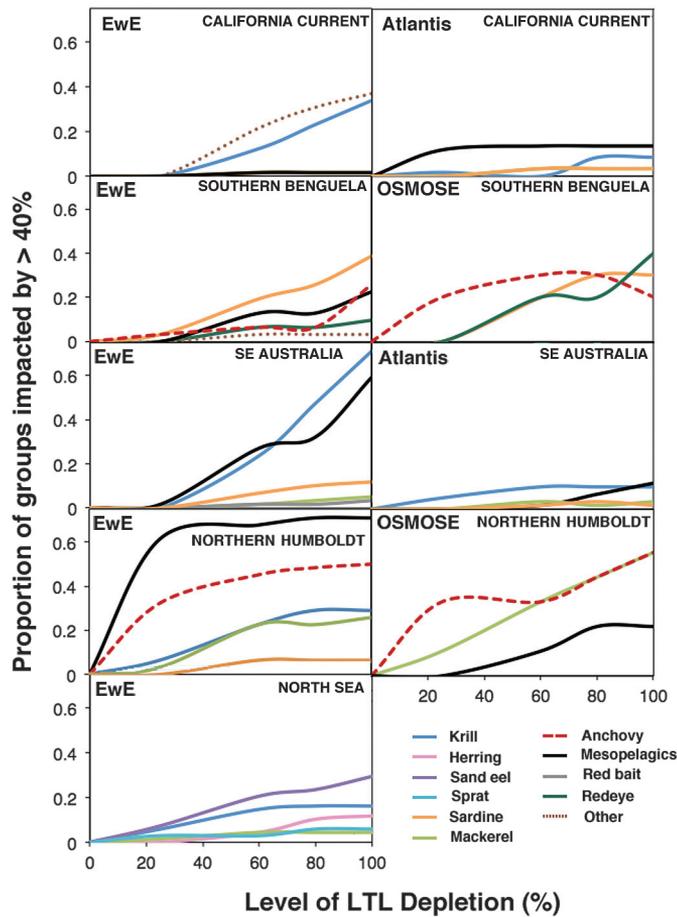

Fig. 3. Relationships between attributes of depleted LTL species and their ecosystem impact. Impacts are scored as the rank of the largest effect: rank 1, no change greater than 20% in any other ecological group; rank 2, no change greater than 60% in any other ecological group; and rank 3, change greater than 60% in at least one other ecological group. Each point corresponds to one ecosystem, model, and LTL species. All LTL species are depleted by 60%. (**A**) Impact of relative biomass of LTL species (biomass as a percent of total consumer biomass in the ecosystem) on rank of largest effect. (**B**) Impact of connectance (proportion of all ecosystem trophic links involving the LTL species) on rank of largest effect.

Fig. 4. Tradeoff between yield and ecological impact as level of LTL depletion varies. Yield (blue) is shown as a proportion of MSY. Ecological impact (gray) is measured as the proportion of other ecological groups whose biomass varied by more than 40%. Shaded zones show ±1.96 times SE. Results are for all ecosystems, models, and LTL species.
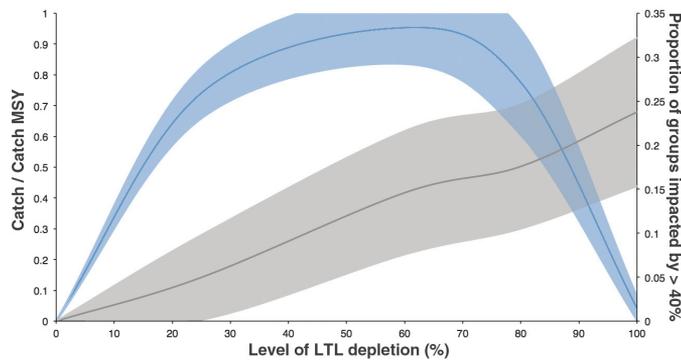
commercially targeted species (fig. S1). Although we did not explicitly examine multi-species harvest strategies, exploitation rates well below MSY levels are consistent with previous findings that lower exploitation rates should be adopted for most species (2).

These results are based on model predictions. Each of the models has been validated against time-series data from well-studied systems, and additional empirical validation for impacts on seabirds and marine mammals is provided in (22). Clearly, the details of which groups respond to depletion of LTL species is sensitive to both model parameterization and to choice of model structure (22). For this reason, we do not consider that these models should be used to determine tactical management decisions. However, the overall findings reported here are robust to details of model choice.

The conclusion that lower exploitation rates are needed for forage species also finds support from a wider set of model types (23). Spatial structure in marine ecosystems is an important factor in species interactions, and local prey depletion may be particularly important for land-based predators such as penguins and seals (24). Two of the models used in this study (OSMOSE and Atlantis) incorporate spatial structure, but not always at the resolution needed to address such issues. All of the models incorporate environmental forcing and variability, which is also an important feature driving the dynamics of many LTL species (25, 26). The finding that connectance influences which species are likely to have larger impacts is potentially important, but although the measure of connectance is easily derived in models, it may be more difficult to determine empirically (and the empirical validity of the indicator would need verification). Previous studies have shown that the ways in which species are connected in the food web can influence system properties (27, 28). Previous studies have also emphasized that additional protection may be needed for forage species (29).

The exploitation patterns examined in this study have involved constant fishing mortality rates. Initial explorations of other forms of exploitation, including use of biomass thresholds

or "set asides" (biomass levels below which no exploitation will occur), suggest that lower ecological impacts could be achieved for similar long-term average yields, but at the cost of higher year-to-year variation in catches. Use of such set asides is already a feature of some LTL fisheries, including a 5-million-ton-minimum spawning stock biomass level for Peruvian anchovy (30) and 150,000 tons for California sardine (31). Closed areas are also used in some fisheries so as to reduce impacts on predators, such as closures for sand eels in some parts of the North Sea to improve the breeding success of sea birds (32).

Although harvest strategies for LTL species vary widely, many stocks are currently fished at levels below the biomass that achieves MSY (22). The results of this study combined with set asides and targeted spatial closures should help inform harvest strategies that achieve ecological objectives while ensuring ongoing substantial yields from LTL groups in support of the ongoing challenge of feeding the global human population (33).

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