Sustaining Fisheries Yields Over Evolutionary Time Scales

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Fishery management plans ignore the potential for evolutionary change in harvestable biomass. We subjected populations of an exploited fish (*Menidia menidia*) to large, small, or random size-selective harvest of adults over four generations. Harvested biomass evolved rapidly in directions counter to the size-dependent force of fishing mortality. Large-harvested populations initially produced the highest catch but quickly evolved a lower yield than controls. Small-harvested populations did the reverse. These shifts were caused by selection of genotypes with slower or faster rates of growth. Management tools that preserve natural genetic variation are necessary for long-term sustainable yield.

It is well established that wild pest and pathogen populations may evolve in response to anthropogenic forces of mortality (*1*), but is the same true of fisheries? Fishing mortality is highly selective. Exploited stocks typically display greatly truncated size and age distributions that lack larger and/or older individuals (*2–4*). This occurs not only because fishers may seek to exploit large individuals but also because regulatory measures often impose minimum size or gear regulations that ensure selective harvest of larger fish. Such harvesting practices could favor genotypes with slower growth, earlier age at maturity, or other changes that would lower population productivity. Despite mounting evidence of rapid life history evolution in wild fish populations (*5–8*), the unexpectedly slow recovery of populations from overexploitation (*9*, *10*), and warnings from theorists (*3*, *11*), current models and management plans for sustainable yield ignore the Darwinian consequences of selective harvest.

Failure to consider evolutionary processes in fisheries management continues in part because proof that size-selective mortality causes genetic changes in population productivity is lacking. Here, we present results from experimentally harvested captive populations of a marine fish that demonstrate evolutionary effects of size-selective mortality on somatic growth, yield, and population biomass.

The Atlantic silverside, *Menidia menidia*, is a common marine fish along the North American east coast. Although landed commercially (mean annual landings in New York, from 1996 to 2000, were 20.5 metric tons), we chose this species as a model primarily for two other reasons. First, many of its life history characteristics are similar to those of other harvested marine species [e.g., high fecundity, small egg size (1 mm in diameter), external fertilization, spawning en masse, pelagic larvae, and schooling behavior], with one major exception. The short generation time of *M. menidia* (1 year) coupled with the ease with which large populations can be maintained in captivity enable experimental designs that would otherwise be impossible. Second, *M. menidia* from different latitudes display clinal adaptive genetic variation in somatic growth rate (*12*), a geographical pattern common to other harvested species (*13–16*). Hence, a key production trait (somatic growth rate) appears capable of evolving in the wild in these species.

We hypothesized that somatic growth rate and population levels of harvest would evolve in directions opposite to the size bias of harvest. To test this premise, we founded six captive populations of *M. menidia* by sampling randomly from a large, common gene pool of embryos produced by mass spawnings of adults collected from the middle portion of the species' range. After the larval phase was completed, 1100 juveniles from each population were stocked in large tanks and reared to the adult stage. Allowing for 10% mortality during the juvenile phase, this resulted in about 1000 fish available for harvest per population. On day 190 postfertilization, 90% of each population was harvested on the basis of one of three different size-specific rules: (i) in two populations, all fish larger than the 10th percentile in length (i.e., the largest 90%) were harvested (large-harvested); (ii) in two other populations, all fish smaller than the 90th percentile (the smallest 90%) were extracted (small-harvested); and (iii) two populations were controls in which 90% harvest was random with respect to size (random-harvested). Survivors ($n \approx 100$) were induced through photoperiod manipulations to spawn, and their embryos were collected and reared under identical conditions over multiple generations (see details of our methods in the supporting online material).

Cross-generation trends in yield of the harvested populations strongly supported our hypothesis (Fig. 1). Large-harvested populations initially produced the highest total yield and mean weight of fish but then declined. Small-harvested populations started with low yield and then increased. By the fourth generation of selection, the biomass harvested and the mean weight of harvested individuals in the small-harvested lines was nearly twice that of the large-harvested lines. Moreover, the spawning stock biomass differed even more. The mean weight of individual spawners (i.e., the survivors) in generation 4 was 1.05, 3.17, and 6.47 g in the large-, random-, and small-harvested populations, respectively. Hence, because fecundity increases with size, small-harvested lines evolved much higher reproductive potential than did large-harvested lines.

The reason for the opposite shifts in yield among the three treatments was genetic change in somatic growth rate rather than viability. Juvenile survival rates differed little among the populations, averaging 83.5, 84.4, and 87.9% in the large, small, and random lines, respectively. Hence, size selection did not merely sort fish with generally favorable or unfavorable genes. Population-level differences in biomass were achieved by increased juvenile growth rates in small-harvested populations and decreased juvenile growth in large-harvested lines (Fig. 2). In

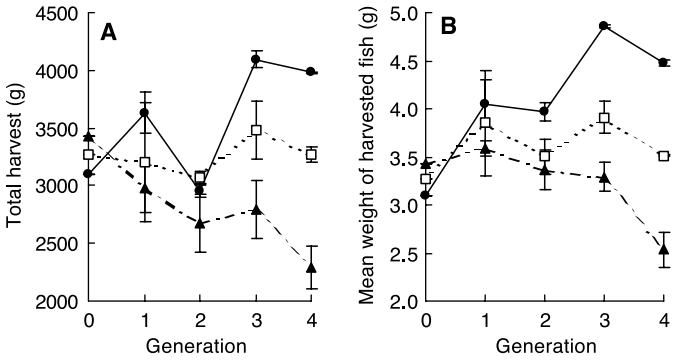

Fig. 1. Trends in average total weight harvested (**A**) and mean weight of harvested individuals (**B**) across multiple generations of size-selective exploitation. Closed circles represent small-harvested lines, open squares are the random-harvested lines, and closed triangles are the large-harvested lines. Each datum is the mean, and the vertical lines show the range of two replicate populations per treatment. Regression analyses showed that both total weight and mean weight harvested declined significantly in the large-harvested lines (slope = −0.82, SE = 0.20, $P = 0.004$; slope = −0.75, SE = 0.23, $P = 0.01$, respectively), increased significantly in small-harvested lines (slope = 0.67, SE = 0.26, $P = 0.03$; slope = 0.83, SE = 0.19, $P = 0.002$, respectively), and did not change in random-harvest lines (slope = 0.13, SE = 0.35, $P = 0.70$; slope = 0.21, SE = 0.34, $P = 0.55$, respectively).

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quantitative genetic terms, the response to selection on size at day 190 was symmetrical, displaying a realized heritability of about 0.2 in both upward and downward directions (Fig. 3).

In addition to growth, other life history traits changed that may also influence population dynamics in nature. Egg sizes were significantly smaller in the large- than in the small-harvested lines [generation 4: mean egg volumes were 0.61, 0.65, 0.72 mm^3 in large-, random-, and small-harvested lines, respectively; nested analysis of variance, $F(2, 6) = 22.7$, $P = 0.002$], which may affect embryo quality and viability. Larval growth rates evolved in parallel—large-harvested populations evolved slower larval growth than did small-harvested lines (Fig. 4). In nature, slower growth would lengthen larval duration, perhaps leading to increased risk of predation or other sources of larval mortality (*17*, *18*). Work in progress suggests that growth-rate differences result from changes in per capita rates of food consumption. Hence, selection on adult size caused the evolution of a suite of traits likely to influence population growth rate and productivity (*19*).

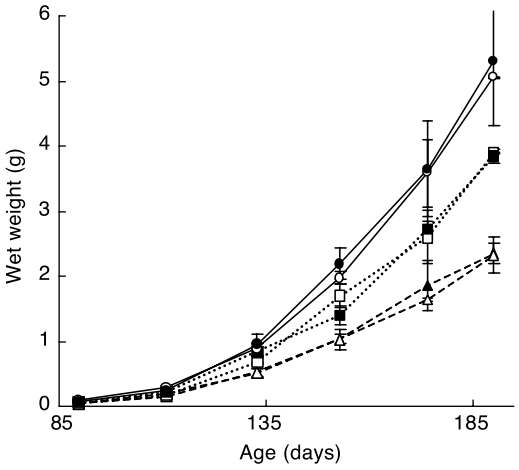

Fig. 2. Trajectories of mean individual weight at age for each of the six harvested populations during generation 4. Circles, squares, and triangles represent the small-, random-, and large-harvested populations, respectively. Open and closed symbols are the two replicate populations within each harvest treatment. Each datum represents the mean wet weight of subsamples of fish from each duplicate phase of each population. Because variance in size increases with age, we increased the number measured in increments of 5 from $n = 15$ on day 90 to $n = 40$ on day 190. Vertical lines represent the range of the duplicate mean weights from each population at each age.

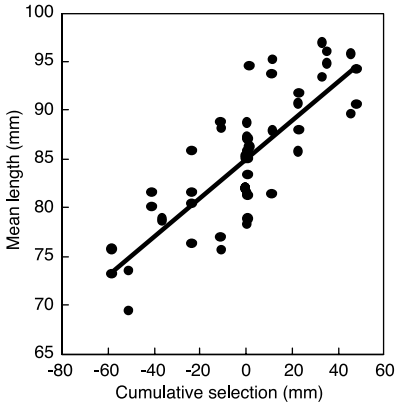

Fig. 3. Heritability (the proportion of trait variance in parents inherited by offspring) of mean length on day 190 (L_{190}). Heritability was estimated using standard methods of quantitative genetics (*28*). Specifically, the model was given by $L_{190} = \mu + X\beta + h^2S + \varepsilon$, where X is the design matrix coding for effects of phase and generation, h^2 is the heritability, S is the vector of cumulative selection differentials (the sum change in mean phenotype of parents caused by selection), and ε is the error term, which was assumed to be normally distributed with a mean of 0. Standard errors for heritability estimates were corrected for drift and sampling error using formulas derived by Hill (*29*). Because each generation of each population was raised in two phases that were selected separately, then pooled before spawning, S is given by the mean selection differential applied to each phase, weighted by the number of parents from each phase. The figure shows L_{190} corrected for generation and phase effects, plotted against the cumulative selection differential. The slope of the least-squares regression through these data is an estimate of the heritability equal to 0.198 ± 0.02 SE.

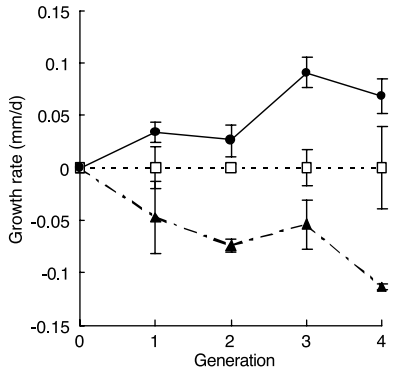

Fig. 4. Rates of larval growth in small- (closed circles) and large-harvested (closed triangles) populations relative to random-harvested controls (open squares). Each generation, the rate of larval growth at 21°C was measured by stocking 30 15-day-old larvae from each population into 19-liter polyethylene containers supplied twice daily with live *Artemia* nauplii ad libitum until age 45 days. There were three to four replicates per generation and line. Initial mean length was estimated from 10 to 15 fish killed at the outset of each trial. Growth rate of each replicate was calculated as [(mean length on day 45) – (mean length on day 15)]/30. The deviations of each of the treatment means from the mean growth rate of the controls are plotted. Vertical lines represent the range of values for the two replicate populations of each treatment. Regression analyses showed that the rate of larval growth increased significantly with generation in the small-harvested lines (slope = 0.79, SE = 0.21, $P = 0.006$) and decreased significantly with generation in the large-size-harvested lines (slope = −0.80, SE = 0.21, $P = 0.006$).

Our empirical model is obviously a simple one. Rates of evolution in captive populations of an annual species under controlled conditions may not be directly comparable to the likely rates of evolutionary change in nature where environmental variability, overlapping generations, and longer generation times of most stocks would reduce the efficiency of, and increase the time required for, response to selection on size. Several lines of evidence suggest that evolutionary responses like those described here are likely to occur in the wild. First, a heritability of 0.2 is typical of life history traits (*19*), and lab-based estimates compare favorably to those from the field in many organisms (*20*), including fishes (*21*). Given evidence of rapid life history evolution of fish in the wild (*5–8*), the potential for evolution in *M. menidia* is not exceptional. Second, the existence of adaptive genetic variation in growth among diverse taxa (*12–16*) proves that production traits like growth are capable of evolving in the wild. Third, although the selection differentials we imposed were severe, those imposed by fisheries are themselves substantial (*22*), with rates of fishing mortality often exceeding natural mortality by a factor of 2 to 3, and with stocks displaying greatly truncated size and age distributions, as compared with pre-exploitation conditions (*2–4*). Fourth, although the generation time of *M. menidia* is short, many longer-lived wild stocks have been harvested for tens or hundreds of generations, which is ample time for evolution.

In wild exploited populations, increased growth resulting from lower fish density may at first obscure the genetic response to selection, unlike in our experiments where density was standardized. Nonetheless, there are well-documented cases where size at age has declined over time in response to fishing (*8*, *23–25*), and over-harvested stocks frequently rebound slowly when fishing ceases (*9*, *10*). Reduced size at age and failure to rebound are consistent with the evolutionary response demonstrated here.

Our study illustrates how well-intentioned management plans that appear to maximize yield on ecological time scales may have the opposite effect after accounting for evolutionary dynamics. Management plans that ignore the evolutionary consequences of fish-

ing may repeat the lessons learned in attempts to control pests and pathogens (*1*), albeit over a somewhat longer time scale. Moreover, the genetic changes caused by selective harvest may be irreversible; cessation of harvest does not guarantee reverse selection back to the original state (*22*). Ignoring evolutionary consequences of selective harvest contradicts the precautionary approach to resource conservation.

What forms of management might help to reduce or incorporate evolutionary changes due to selective fishing? First, the establishment of no-take reserves or marine protected areas may, if properly designed, provide for the maintenance of natural genetic variation by allowing a portion of the stock to express an unconstrained range of sizes and growth rates (*26*, *27*). Second, reliance on minimum size restriction (all fish below a given size are protected) as a basis for management needs rethinking. Where feasible, maximum size limits (all fish above a given size are protected) may offer some important advantages: (i) fast-growing genotypes that pass more quickly through the period of vulnerability would be favored by selection; (ii) the age structure would broaden, thereby increasing spawning stock biomass; and (iii) the ecosystem services provided by large animals would be restored (*2*). Harvest regimes that account for the Darwinian effects of fishing need serious consideration if yields are to be truly sustainable.

References and Notes

1. S. R. Palumbi, *Science* **293**, 1786 (2001).
2. J. B. C. Jackson *et al*., *Science* **293**, 629 (2001).
3. T. K. Stokes, J. M. McGlade, R. Law, *The Exploitation of Evolving Resources* (Springer-Verlag, Berlin, 1993).
4. K. I. Stergiou, *Fish. Res.* **55**, 1 (2002).
5. D. N. Reznick, H. Bryga, J. A. Endler, *Nature* **346**, 357 (1990).
6. T. P. Quinn, M. T. Kinnison, M. J. Unwin, *Genetica* **112**, 493 (2001).
7. A. P. Hendry, *Genetica* **112**, 515 (2001).
8. T. O. Haugen, L. A. Vollestad, *Genetica* **112**, 475 (2001).
9. J. A. Hutchings, *Nature* **406**, 882 (2000).
10. J. A. Hutchings, *J. Fish Biol.* **59**, 306 (2001).
11. R. Law, *ICES J. Mar. Sci.* **57**, 659 (2000).
12. D. O. Conover, T. M. C. Present, *Oecologia* **83**, 316 (1990).
13. A. K. Imsland, T. M. Jónassen, *Rev. Fish Biol. Fish.* **11**, 71 (2001).
14. C. F. Purchase, J. A. Brown, *Can. J. Fish. Aquat. Sci.* **57**, 2223 (2000).
15. A. G. Nicieza, F. G. Reyesgavilan, F. Braña, *Can. J. Zool.* **72**, 1603 (1994).
16. D. O. Conover, J. J. Brown, A. Ehtisham, *Can. J. Fish. Aquat. Sci.* **54**, 2401 (1997).
17. K. M. Bailey, E. D. Houde, *Adv. Mar. Biol.* **25**, 1 (1989).
18. E. D. Houde, *J. Fish Biol.* **35**, 29 (1989).
19. D. A. Roff, *Life History Evolution* (Sinnauer Associates, Sunderland, MA, 2002).
20. I. Weigensberg, D. A. Roff, *Evolution* **50**, 2149 (1996).
21. J. Jónasson, B. Gerde, T. Gjedrem, *Aquaculture* **154**, 219 (1997).
22. K. Stokes, R. Law, *Mar. Ecol. Prog. Ser.* **208**, 307 (2000).
23. W. E. Ricker, *Can. J. Fish. Aquat. Sci.* **38**, 1636 (1981).
24. P. J. Harris, J. C. McGovern, *Fish. Bull.* **95**, 732 (1997).
25. Y. Chen, L. G. S. Mello, *Fish. Res.* **42**, 87 (1999).
26. J. Trexler, J. Travis, *Bull. Mar. Sci.* **66**, 853 (2000).
27. National Research Council, *Marine Protected Areas: Tools for Sustaining Ocean Ecosystems* (National Academy Press, Washington, DC, 2001).
28. D. S. Falconer, *Introduction to Quantitative Genetics* (Longman, New York, ed. 2, 1981).
29. W. G. Hill, *Biometrics* **28**, 747 (1972).

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Supporting Online Material

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Materials and Methods

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An Essential Role of N-Terminal Arginylation in Cardiovascular Development

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The enzymatic conjugation of arginine to the N-termini of proteins is a part of the ubiquitin-dependent N-end rule pathway of protein degradation. In mammals, three N-terminal residues—aspartate, glutamate, and cysteine—are substrates for arginylation. The mouse *ATE1* gene encodes a family of Arg-tRNA-protein transferases (R-transferases) that mediate N-terminal arginylation. We constructed ATE1-lacking mouse strains and found that *ATE1*$^{-/-}$ embryos die with defects in heart development and in angiogenic remodeling of the early vascular plexus. Through biochemical analyses, we show that N-terminal cysteine, in contrast to N-terminal aspartate and glutamate, is oxidized before its arginylation by R-transferase, suggesting that the arginylation branch of the N-end rule pathway functions as an oxygen sensor.

Substrates of the ubiquitin (Ub)–dependent N-end rule pathway include proteins with destabilizing N-terminal residues (*1*–*4*). A set of amino acids that are destabilizing in a given cell yields a rule, called the N-end rule, that relates the in vivo half-life of a protein to the identity of its N-terminal residue (*1*–*3, 5*–*8*). The N-end rule has a hierarchic structure. Specifically, N-terminal Asn and Gln are tertiary destabilizing residues in that they function through their deamidation, by N-terminal amidohydrolases (*7*), to yield the secondary destabilizing residues Asp and Glu, whose activity requires their conjugation, by *ATE1*-encoded Arg-tRNA-protein transferases (R-transferases) (*5*), to Arg, one of the primary destabilizing residues. The latter are recognized by the Ub ligases (E3 enzymes) of the N-end rule pathway (Fig. 1A) (*3, 4, 9*).

In mammals, the set of destabilizing residues that function through their arginylation includes not only Asp and Glu but also Cys, which is a stabilizing (nonarginylated) residue in the yeast *Saccharomyces cerevisiae* (*5, 10, 11*). ATE1-1 and ATE1-2, the isoforms of mammalian R-transferase, are produced through alternative splicing of *ATE1* pre-mRNA and have the same specificity as the yeast R-transferase: They arginylate N-terminal Asp or Glu but not Cys (*5*). This raises the question of how N-terminal Cys is arginylated in mammalian cells. To address this issue and the physiological functions of arginylation, we constructed *ATE1*$^{-/-}$ mouse strains (*12*).

Whereas *ATE1*$^{+/-}$ mice were apparently normal, the *ATE1*$^{-/-}$ genotype conferred embryonic lethality (*12*). The *ATE1*$^{-}$ allele was marked with NLS-β-galactosidase (βgal) (*12*). During embryonic day (E) 9.5 to 12.5, the expression of βgal was high in the neural tube and other specific (often sharply delineated) regions of developing embryo (*12*). *ATE1*$^{-/-}$ embryos were pale and had thinner blood vessels and frequent edemas of the skin (Fig. 1, B and C; Fig. 2, A and B) (*12*). Hemorrhages were a consistent feature of *ATE1*$^{-/-}$ embryos and were the likely proximal cause of their death (Fig. 1, D and E). Of 22 *ATE1*$^{-/-}$ hearts (E13.5 to E15.5) examined, ~85% had a ventricular septal defect (VSD) (Fig. 1, I and J). The atria of *ATE1*$^{-/-}$ hearts were thin walled, with sparse trabeculae and a large atrial septal defect (ASD) (Fig.

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