Multigenerational exposure to warming and fishing causes recruitment collapse, but size diversity and periodic cooling can aid recovery

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Global warming and fisheries harvest are significantly impacting wild fish stocks, yet their interactive influence on population resilience to stress remains unclear. We explored these interactive effects on early-life development and survival by experimentally manipulating the thermal and harvest regimes in 18 zebrafish (*Danio rerio*) populations over six consecutive generations. Warming advanced development rates across generations, but after three generations, it caused a sudden and large (30–50%) decline in recruitment. This warming impact was most severe in populations where size-selective harvesting reduced the average size of spawners. We then explored whether our observed recruitment decline could be explained by changes in egg size, early egg and larval survival, population sex ratio, and developmental costs. We found that it was most likely driven by temperature-induced shifts in embryonic development rate and fishing-induced male-biased sex ratios. Importantly, once harvest and warming were relaxed, recruitment rates rapidly recovered. Our study suggests that the effects of warming and fishing could have strong impacts on wild stock recruitment, but this may take several generations to manifest. However, resilience of wild populations may be higher if fishing preserves sufficient body size diversity, and windows of suitable temperature periodically occur.

climate change | fishing | evolution | reproduction | recruitment

Global warming and harvesting are causing rapid and drastic changes to many of the Earth's ecosystems (1, 2). These impacts are particularly prevalent in the aquatic realm where harvesting of wild fish populations can greatly exceed rates of natural mortality and where, due to the physical nature of the aquatic medium, individuals cannot readily escape warming through behavioral modulations or habitat choice (3, 4). The additive effects of warming and fishing on population size structure, reproductive output, and recovery potential post disturbance are well known (3, 5–7). Of particular concern, however, is the interaction of these forces and how they together might impact global fisheries' sustainability. Empirical studies suggest that harvesting can amplify a fish stock's sensitivity to environmental changes (8–11), but the mechanisms underpinning these effects, and their potential reversibility, often remain unclear.

Global warming can affect fish populations by causing changes in egg size, early development, and recruitment success (12). For example, development cost theory suggests that species' development and metabolic processes during the egg to feeding stages respond to changing temperatures at different rates (13). These differential rates determine an optimum temperature for a species' development, which occurs when metabolism is relatively low, development is sufficiently fast, and thus the total cost of development is minimized (13). As temperature cools or warms, respectively longer development times or higher metabolic rates lead to faster use of energy reserves, poorer larval condition upon hatching, and potentially negative effects on recruitment (13, 14). Likewise, later in life, higher temperatures may lead to increased metabolic rates and energetic expenditure during maturation, which in turn can alter energetic allocation to progeny (15) and thus egg size and larval condition (16).

Another way that warming might affect fish stocks is through changes in the size structure of populations (e.g., refs. 17 and 18). This effect will be particularly strong where population size structure is also impacted by harvesting (8). Moreover, warming- and fishing-induced changes in size structure can be driven not only by the direct removal or elevated mortality of large individuals, but also through plastic (e.g., temperature-size rule: refs. 19 and 20), intergenerational (maternal effects; e.g., ref. 21), and evolutionary responses (e.g., fisheries-induced evolution: ref. 22). Modified size distributions could in turn impact on stock resilience by reducing size-related reproductive output (23), population "storage effects" conferred by large individuals (24), or shifting size-related ecological interactions (25, 26). Changes in size distributions can thus increase the environmental sensitivity of populations and lead to biomass fluctuations (8, 11).

Despite numerous studies focusing on how fishery stocks respond to warming or harvesting, a major question remains unclear: How do these two forces interact to impact on stocks over long-term, intergenerational timescales? Through experimentation, we know that fishes respond differently to acute or short-term warming compared to multigenerational exposure (27–29) and that fishing selection can drive long-term trait changes (22, 30). Here, we address this knowledge gap by exploring the long-term

Significance

The synergistic impacts of rapid climatic warming and fisheries harvest are threatening the sustainability of wild fisheries. Their collective impact on fish recruitment—a key process underpinning stock abundance—remains poorly understood. We experimentally exposed fish populations to realistic warming and fishing-selection regimes over multiple generations and found that warmed populations experienced a severe decline in recruitment rate. This warming-induced decline was exacerbated by size-selective fishery harvest. Once warming and size-selective fishing were relaxed, recruitment rates rapidly recovered. Our results suggest that synergistic impacts of fishing and warming can have delayed effects on stock resilience and that preserving fish body size diversity will help to increase their resilience to global warming.

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interactions of harvest and warming on the reproductive output, early life history, and recruitment of fish populations.

Using a multigenerational selection experiment on the tropical freshwater zebrafish (*Danio rerio*), we exposed 18 independent populations to factorial combinations of two temperature treatments (control, 26 °C, and "hot," 30 °C) and three size-based fisheries selection regimes and then measured a range of early life characteristics. Populations were acclimated for two generations to minimize any maternal effects (31), after which we imposed five generations of warming and fishing selection followed by two generations of common-garden conditions with control temperatures and random size harvesting. Our hot treatment was at the upper end of temperatures experienced by zebrafish in the wild (32), while our fisheries selection regimes imposed high but realistic levels of mortality (80% harvest imposed once the majority of individuals are mature, e.g., ref. 33) and realistic trawl and gillnet fisheries selectivity curves (sigmoid, Gaussian, and random control harvesting) (*SI Appendix*, Fig. S1). Our selectivity regimes were also more reflective of real-world conditions than some previous multigenerational experiments, where knife-edge selectivity was used (22, 30, 34, 35). Our temperature treatments span a realistic, short-term warming scenario (36). With this design, we tested whether there is a significant interaction of warming and size-selective fishing on reproductive output (egg size and egg size variation), early life history (survival and development rate), and survival to subadult stage, which approximates "recruitment" of wild stocks. Together, these results help identify the mechanisms underpinning fish population responses to the combined impacts of fishing and warming and facilitate prediction of population states in a warmer future with increased demand on wild fisheries (26).

Results

We found a strong synergistic effect of warming and fishing on fish survival to the subadult stage (week 7 postspawn), which is equivalent to recruitment in a wild fishery. Recruitment rates in wild populations are the outcome of spawning- and development-related processes (37), as well as the environmental context (38), and indicate a population's capacity to provide new individuals into the breeding or harvestable fraction of a stock (37, 39). The age at which individuals "recruit" is fishery dependent but often represents those close to the maturation size as occurred in our experiment. Recruitment in wild stocks is often regulated by the

environmental carrying capacity and therefore capped at some maximum level. Here, we defined recruitment in terms of the proportion of the aquarium carrying capacity (250 individuals per population by week 7; *Materials and Methods*).

All of our populations had a very high recruitment rate over the first three generations. In the fourth generation, recruitment rates suddenly declined by ~30% in hot populations, and then even further (~50%) in the fifth generation where one population had a complete reproductive failure (Fig. 1*A* and *SI Appendix*, Table S2). The biggest decrease and largest among-population variation in recruitment occurred in hot populations exposed to trawl-like fisheries selectivity (sigmoid selection curve), where larger individuals were harvested and only smaller fish were left to reproduce. For these sigmoid selection populations, recruitment at 30 °C in the F5 generation was ~21% lower than the corresponding gillnet type (Gaussian) and random size selected populations (Fig. 1*B* and *SI Appendix*, Table S2). This reproductive failure was unlikely to be caused by recruitment overfishing, such that populations below some threshold (e.g., adult density) cannot supply enough propagules (40), as all of our fishing selection treatments were designed to preserve at least 40 adult spawners. Furthermore, cold populations exposed to the same fishing intensity did not suffer recruitment collapse which suggests processes aside from adult density were at play. Recruitment did recover immediately and fully in the remaining populations in the first common garden generation (CG1), when the developmental temperature of F5 eggs spawned at 30 °C was gradually returned to control conditions (26 °C) for further egg development (Fig. 1*A* and *SI Appendix*, Table S2). This full recovery persisted into the second common generation (CG2), where all populations had full recruitment (Fig. 1*A*). There are several potential reasons for this delayed, but strong, impact of warming and harvesting, and subsequent rapid recovery, on zebrafish recruitment, and we explored four of them: 1) changes in egg size and quality (41), 2) changes in early egg and larval survival (42), 3) changes in population sex ratio (43), and 4) developmental costs (13).

To assess whether warming and harvesting affected egg size and provisioning, and in this way impacted on the recruitment rate of F4 and F5 generations, we measured the size of 5,367 egg yolks from four treatment generations. We measured yolk rather than total egg size because it better reflects egg energetic content and thus maternal investment (44). There was no evidence of an
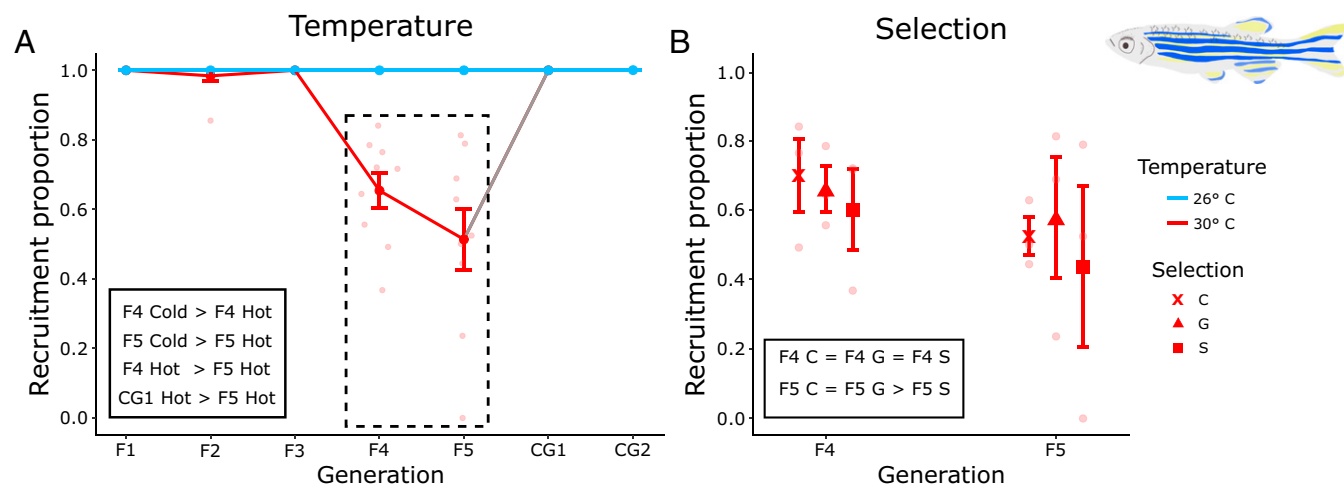
Fig. 1. Effects of temperature (*A*) and size-based harvesting (*B*) on recruitment proportion (Pr.) across generations. Recruitment rate is defined as the proportion of the carrying capacity of our system (*Materials and Methods*). Statistical comparisons among treatment groups are shown on each panel. Note: hot populations were reared at 26 °C in the common garden generations (indicated by the gray line in *A*). The data in *B* come from the dashed area in *A*, with selection treatments denoted by C, control; G, Gaussian; and S, sigmoid. Transparent points in *A* and *B* represent raw data. The solid symbols show means ± SE.

interactive effect of warming and size-selective harvest on egg size. We did, however, find that hot-treatment mothers produced eggs with yolks that were on average 4% larger in diameter (~11% in volume) than those from control temperature (26 °C) fish (Fig. 2 and *SI Appendix*, Tables S3 and S4). This temperature-induced difference in egg size persisted across generations and was not explained by the length of spawners (mean length and weight of spawners at 26 vs. 30 °C: 39.6 vs. 39.1 mm; 0.86 vs. 0.76 g; *SI Appendix*, Tables S5 and S6). Thus, we did not find evidence that egg size has changed through generations and could explain low survival in F4 and F5.

Our observation of larger egg size at warmer temperatures contrasted with the expectation that mothers inhabiting warmer environments should produce smaller eggs (23, 45). Instead, our results were consistent with a recent hypothesis predicting that optimal egg size should follow a nonlinear pattern across temperature within species (13). Here, eggs are expected to be smallest at temperatures where an interaction of increasing metabolic rate and decreasing developmental time produce minimal developmental costs during the yolk-dependent nonfeeding phase (13). Increased maternal provisioning at 30 °C (compared to 26 °C) in our experiment could thus be a maternal response to ensure sufficient energy reserves are available to offset higher embryonic developmental costs.

Furthermore, egg size and the coefficient of egg size variation did not clearly differ across fishing treatments (*SI Appendix*, Tables S3 and S4), despite the average spawner size being smaller in sigmoid (0.65 g) compared to Gaussian (0.97 g) and control (0.82 g) selection populations (*SI Appendix*, Tables S4 and S5). This contrasts with other studies linking egg size to fisheries harvest (22), and instead suggests that either zebrafish mothers across the maternal size spectrum produced similarly sized eggs, or that only a subset of equally sized mothers across fishing treatments spawned in each generation. We did not have access to individual spawning data and could not separate between these two alternatives.

Given that yolk size could not clearly explain the rapid decrease in recruitment in the F4 and F5 generations, we then looked at whether there was an interactive impact of warming and fishing on early survival, as the early life stages of fish are highly

sensitive to the effects of warming (12, 46). We measured egg and hatched larvae survival to day 3 after fertilization in the F1–F5 and common garden generations (*Materials and Methods*) but found no generation effect and no interactive effect of warming and harvest (*SI Appendix*, Table S3). Early survival was slightly reduced in warmer populations (across all generations), but only when eggs were housed at high density, likely reflecting the impacts of random variation in experimental conditions (*SI Appendix*, Fig. S8 and Tables S3 and S4). Therefore, recruitment collapse in generations F4 and F5 for hot populations could not be explained by low early survival.

Next, we investigated whether warming and fishing could have affected zebrafish sex ratios in our populations, and whether this led to the observed recruitment failure. Male-skewed sex ratios could be induced by fishing, because zebrafish females are generally larger than males and thus more vulnerable to trawl-like selectivity (for a similar example on how fishing can affect sex ratio in salmon, see ref. 43). Furthermore, warmer developmental temperatures can also produce more males in zebrafish (47). We analyzed the sex ratios of all spawners in the F2 and F4 generations as these fish led to either successful (F3) or very low (F5) recruitment. Overall, there was a slightly higher proportion of males in the F2 and F4 hot Gaussian populations and the hot F4 sigmoid population. However, there was no temperature-dependent difference in random selection treatments, despite hot randomly selected populations also having low recruitment in the F5 generation (*SI Appendix*, Fig. S9 and Tables S3 and S4 for model results). Notably, the one population that completely failed to reproduce in the F5 generation (hot treatment and sigmoid selectivity) did have the highest observed proportion of males (87%). These findings suggest that a combination of size-selective fishing and warming might affect population sex ratios and could contribute to low reproductive output and recruitment. Such a phenomenon would be especially concerning in species where sex determination is temperature dependent (48). Nonetheless, skewed sex ratios did not fully explain failed recruitment in our last experimental generation. Reduced recruitment was observed in all F5 hot treatments, including the random size selection treatment, where sex ratios remained close to 1:1 across generations. These results indicate that other factors might also be at play, and to investigate them we explored developmental rates across generations.

Low recruitment success in hot populations could be at least partly explained by the developmental cost theory and its implications (13). If optimal developmental temperature is indeed determined by a fine balance between developmental and metabolic rates, as the theory suggests, then individuals developing at suboptimal temperatures may have lower condition and hence lower long-term survival. Our two temperature treatments (26 and 30 °C) sit either side of the suggested optimal developmental temperature for zebrafish (~28 °C: ref. 13) at which our parental generations were housed. If an increase in metabolic rates at 30 °C is not offset by a commensurate decrease in developmental time, then the cost of development would increase. Due to the exponential nature of temperature response curves (49), this cost would be more pronounced for the hot populations (30 °C treatment), which were also close to the maximum observed temperatures for wild zebrafish (32). The observed larger egg yolk sizes at 30 °C support this argument as they suggest that females increase energy provisioning to their offspring to account for relatively higher developmental costs in warmer water. It is then logical to assume that development at suboptimal temperatures could reduce long-term survival and have fitness consequences. This is hard to assess properly in experimental populations that are not exposed to predation, food scarcity, and environmental fluctuations that affect wild stocks. However, even in experimental conditions, we could ask whether long-term exposure to suboptimal temperatures can affect developmental rate, which might help to reduce developmental costs?
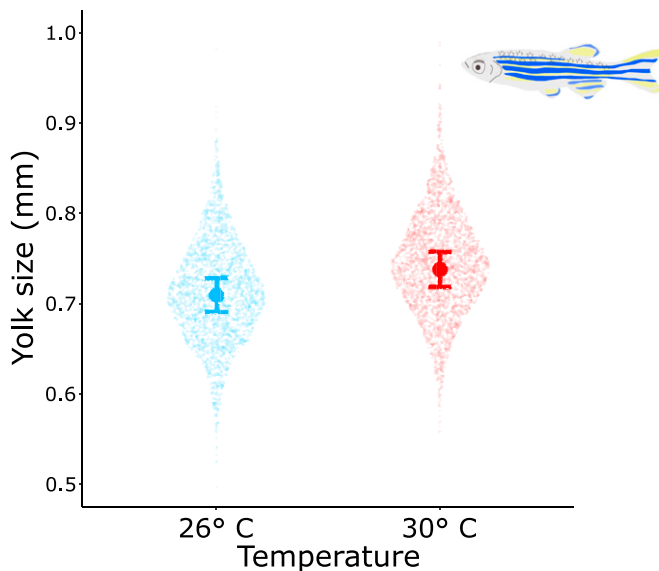
Fig. 2. Model-predicted egg yolk size (yolk diameter in millimeters ± 95% CI) for control and warm temperature treatments, after accounting for random effects. Transparent points show raw data where the spread of data along the *x* axis indicates data density.

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To answer this question, we measured embryonic development rate (time to 50% hatch) and indeed found that the difference between the two temperature treatments increased across generations. As expected, across all generations, eggs at 30 °C hatched earlier than eggs at 26 °C (Fig. 3). However, in the F1 generation, hot eggs hatched on average 32% earlier, and by the fifth generation (F5) this difference had increased to 38% (Fig. 3*A* and *SI Appendix*, Tables S3 and S4). In the first common garden generation (CG1), development rate in previously hot populations remained slightly faster than in control treatments, although the difference was no longer significant (Tukey adjusted pairwise comparison, df = 16.1, *t* ratio = −1.872, *P* = 0.278, Fig. 3*B*). We also observed a slow decrease in development rate at the control temperature of 26 °C, and this decrease continued into the common garden period. Such slowing down of development could suggest optimization of developmental rate to 26 °C, or general adaptation to other laboratory and experimental conditions, as is commonly observed in multigenerational experiments (50).

Our results suggest that hot treatment populations steadily hastened their developmental rates throughout the five generations. Because the difference in the common garden period quickly became insignificant, we conclude this to be a phenotypic or intergenerational effect rather than evidence for rapid trait evolution. Further genomic analyses are needed to formally test this conclusion (DNA and RNA samples from this experiment are preserved for future analyses). Regardless of the underpinning causal mechanism, our study suggests that faster developmental rates might have survival costs that could have contributed to the decreased recruitment of F4 and F5 generations. In wild populations where early predation is often extreme (51), the costs of faster development could be overcome if the resultant shorter larval phase leads to lower total mortality. While our results cannot separate between different alternative explanations, they do call for further investigation of two key questions: 1) How much does the optimal development temperature (13) vary within a species through space and time and is there plasticity or evolutionary potential to adjust this temperature? and 2) What are the trade-offs between faster early development, expected in warming oceans, and long-term survival?

Conclusion

Our study provides strong evidence that the recruitment capacity of fish populations is acutely sensitive to both warming and harvest, but that these effects may take several generations to manifest. This poses a significant threat to wild populations, as warming-driven reductions in recruitment rates could be exacerbated by intensive fisheries harvest and drive local extinction events. Our findings also show that short-term studies may underestimate the effects of warming and fishing on critical demographic traits. The observed interactive effect of warming and fishing on recruitment could partly be explained by the harvest-driven alteration of sex ratios, where simulated trawl fishing led to male skewed populations. These effects are likely to be especially prevalent in species that are more sexually dimorphic with respect to size (43) and could threaten the long-term survival of species through direct impairment of reproductive potential, or through modifications to sexual behaviors or competition (52). However, sex ratios could not completely explain low recruitment in our study, and we suggest that another alternative mechanism could be related to the optimization of early development rates and costs (13). The quick recovery seen in the first common garden generation shows that the warming and size selective harvest-driven effects on recruitment may be relatively short term and fish populations may possess some resilience to warming, if favorable windows of temperature occur and phenotypic diversity of populations is maintained. Naturally, wild populations are exposed to a considerably higher number of stressors, including multiple heating events, changing species interactions, food availability, environmental stochasticity, and other processes that can lead to depensation and Allee effects (53, 54), which can slow down or completely impede recovery. Moreover, as recovery rate is likely to be driven by factors such as the size or age at maturity and fecundity, other fished species [e.g., elasmobranchs (55) and some species of groundfish (56)] may not respond as quickly. Nevertheless, our study suggests that warming and harvest-driven recruitment collapses might be caused by short-term rather than long-term effects, and further studies are urgently needed to explore potential mechanisms. Most importantly, our findings suggest that preserving the size diversity of fish stocks, through changes in fisheries selectivity (24) or marine protected areas (39), is crucial to improve fisheries resilience in the face of global warming.

Materials and Methods

Overall Experimental Design. Our experiment comprised 18 independent populations (initial *n* = 250 adults each) of zebrafish (*Danio rerio*). All work presented here was conducted under and in accordance with the University of Melbourne's animal ethics application No. 1714101.7. Fish were bred from 2,000 wild-type individuals over two parental generations (PG1 and PG2), where PG1 was held at 28 °C to control for carryover effects from previous housing and breeding conditions, as fish had been held at 28 °C by the supplier. We employed a fully crossed factorial design of two temperatures (control, 26 °C; hot, 30 °C) and three fishing selection regimes (sigmoid selection, Gaussian selection, and random size selection). These were applied
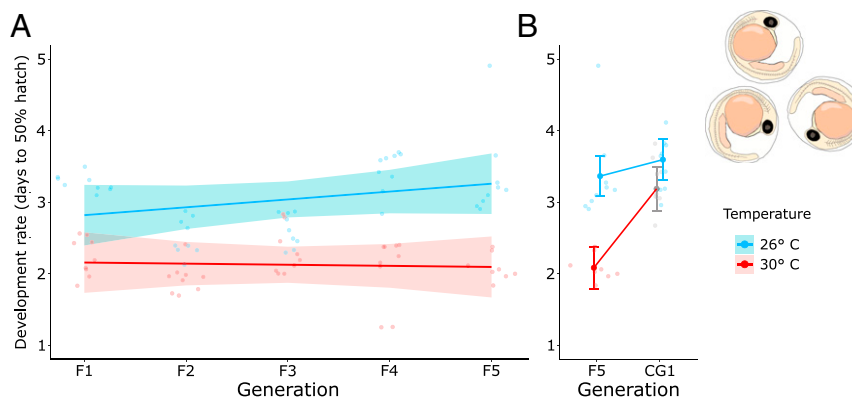
Fig. 3. Model-predicted effects of temperature on development rate (time to 50% hatch) across five treatment generations (*A*), and the first common garden (*B*) generation of zebrafish (*Danio rerio*). Lines in *A* and solid points in *B* represent model predictions (with 95% confidence intervals) (see *SI Appendix*, Tables S3 and S4 for model details). Note that hot populations were incubated at 26 °C in the common garden generation (gray points in *B*). Transparent points represent raw data where the spread of data along the *x* axis indicates data density.

to three replicate populations over five nonoverlapping generations (F1 to F5). Temperature treatments were adjusted from 28 °C in the PG2 generation so that both control and hot populations experienced the same 2 °C temperature change. Subsequent to our F generations, populations were held for two common garden generations (CG1 and CG2) where fishing selection and hot treatments were relaxed back to control levels (26 °C and mortality control). Throughout the experiment, fish were housed in six large (200-L) tanks that were split into three separate sections by mesh dividers to each house one individual treatment population (~70 L per 250 individual population). Three tanks were held at 26 °C and three tanks were held at 30 °C. One replicate population from each size selection treatment was randomly allocated to a section within each tank (see *SI Appendix*, Fig. S4 for a map of our experimental design).

Fishing selection was applied at the end of each generation (PG2-F4) after ≥80% of individuals were sexually mature in 80% of populations, as determined by weekly assays. Sigmoid fishing selection (Eq. **1**) was directional and designed to mimic trawl fishing where larger individuals have an increasing probability of being caught. Gaussian fishing selection (Eq. **2**) was disruptive and designed to mimic gill net fishing or a "slot" limited fishery where the midsized individuals are more likely to be caught. Control selection applied equal probability of capture to all individuals (irrespective of size) and ensured that all populations consisted of an approximately equal number of spawners in each generation. A no-fishing selection control was not included in our experimental design as we were interested in assessing the effects of size-selective harvest rather than investigating the density-dependent effects of fisheries harvest. After selection, the surviving fish were allowed to breed.

When applying selection to each population in each generation, the probability of mortality from fishing at size i (pS_i) was defined as follows:

$$pS_i = \frac{\lambda}{1 + e^{-\frac{(i-\tau)}{2}}},$$ **[1]**

for sigmoid selection,

and

$$pS_i = \lambda * e^{\frac{(i-\bar{S})^2}{2\sigma^2}},$$ **[2]**

for Gaussian selection,

where λ is a population-specific scaling parameter that adjusted the maximum mortality level (i.e., total mortality at targeted lengths), τ is the midpoint of the sigmoid function, $\bar{S}$ is the mean length of Gaussian populations, and σ is the width of the Gaussian selection curve. Selection function parameters were tailored to each population in each selection generation according to its specific size-frequency distribution. This was to ensure selection intensity remained comparable through generations (see *SI Appendix, Supplementary methods* for further details). In sigmoid selection, the midpoint of the selection function was adjusted so that 80% mortality was achieved. Alternatively, the width of the selection function was adjusted in Gaussian selection to apply 80% mortality.

To estimate selection functions, we generated size-frequency distributions by measuring all individuals within a population to the nearest 1 mm (total length) and placing them in 1-mm length bins. Then a proportion of randomly chosen individuals within each length bin were selected against (euthanized), as calculated by the mortality probability at size in Eqs. 1 and 2. Throughout our experiment, 80% of individuals were culled at the selection stage unless poor survival to maturity precluded this mortality rate. In instances of poor juvenile survival, lower selection intensity (mortality) was applied to ensure that 40 breeding individuals were left in the population (see *SI Appendix*, Table S1 for specific mortality rates for populations in each generation). Surviving individuals were held for another week to acclimate to spawning conditions and then spawned over a period of 7 d to initiate the following generation.

Spawned eggs were incubated in Petri dishes at their respective treatment temperature until hatching (2–6 d), whereafter fry were transferred to one of 18 fry-rearing tanks (20 L). Fry were kept in these tanks for 6 wk and then transferred to the main experimental tanks. Throughout the experiment, populations were fed twice daily (morning and afternoon) ad libitum for 10 min and excess food was removed after feeding. Newly hatched fry were fed *Paramecium* spp. for approximately 1 wk, and then *Artemia* spp. nauplii for approximately a month. After that, fish were fed with a commercial pellet food (Nutra Xtreme C1; Aquasonic Pty. Ltd.). Fry and adults were housed on a 12:12-h lighting cycle with constant heating (via aquarium heaters) and filtration (mechanical, biological, and UV). Water quality was monitored each week and adjusted if quality was outside the parameter

range specified in the animal ethics application (1714101.7; The University of Melbourne Animal Ethics Committee). See *SI Appendix* for further details of aquaria setup, animal husbandry, and generational timeline.

Trait Measurements. Across all temperature and size-selective fishing treatments, we measured the sex ratio after spawning (generations F2 and F4), egg yolk size (F2–F5), egg and larval survival to day 3 postfertilization, egg development rate (see below), and survival to week 7, which corresponds to the onset of maturity and transfer to the adult tanks (F1–CG2 generation). Recruit survival was calculated as a proportion of the 250 individuals needed to found adult populations in each generation (*SI Appendix, Supplementary methods*). This effectively created an "upper ceiling" in our dataset where any populations which contained more than 250 recruits upon transfer to adult tanks were also considered as fully survived (i.e., Pr. recruit survival = 1).

A random selection of eggs (number proportional to spawning intensity) was taken from each population, in each generation, on the second day of the 7-d spawning period. Eggs were placed into a 90-mm Petri dish with egg water (a salt solution for raising embryos: see *SI Appendix* for recipe) and photographed daily during incubation using a digital SLR (Nikon D5200 with 40-mm macro lens). Photos of eggs on the day of spawning were taken before the gastrula stage, at which time the blastoderm begins to overgrow the yolk mass and obscure measurement (57). Photos of developing embryos on subsequent days were taken at midday. All image analysis was conducted using the image analysis software ImageJ (version 1.50i: https://imagej.nih.gov/ij). Egg yolk size was measured as the longest axis from each of at least 50 egg's yolk diameter on the day of spawning. Egg yolk size coefficient of variation (CV) for each dish was calculated from these measures of egg yolk size. To investigate spawner size, we also measured the total length (in millimeters) and weight (in grams) of a random sample of 10 individuals from each spawning population 7 d after the spawning event in each generation.

Early survival was estimated as the number of individuals alive (eggs and hatched fry) after 3 d of incubation, as a proportion of live eggs on the day of spawning. The third day postspawning was selected because this was the final day where all populations across generations were still being incubated. Incubation length was dictated by the development rate and some dishes had already been transferred to fry tanks by day 4 (*SI Appendix, Supplementary methods*). Sex ratios were measured at the end of the F2 and F4 generations after fisheries selection, to explore the impacts of size-based harvest on a sexually dimorphic species. The F4 generation was the last to experience fisheries selection. All culled individuals at the end of F2 and F4 were dissected under a microscope and the sex of each individual recorded.

Development rate (predicted time to 50% hatch) was estimated using a four-parameter dose–response model (58), which fitted a logistic function to the hatched proportion of available eggs (calculated as proportion of hatched eggs each day as a function of alive eggs and fry on the preceding day) through time (days). This generated an estimate of relative development rate, which was independent of egg and fry survival and thus allowed for the estimation of development rates in the dishes where high mortality would otherwise obfuscate raw hatch estimation (see *SI Appendix*, Figs. S6 and S7 for model fits to data). Data supporting this manuscript can be found at GitHub (59).

Data Analyses. All data analyses, calculations, and graphical representation were performed using the statistical program R 3.5.1 (60) using the RStudio (61) interface (version 1.3.959) with the additional packages "drc" (58), "lme4" (62), "lmerTest" (63), and "effects" (64, 65). We investigated survival to recruitment using two and three proportion *Z* tests, sex ratios using binomial generalized linear models, and variation in egg yolk size, CV of egg yolk size, early survival, development rate (time to 50% hatch), and spawner size using linear and generalized linear mixed effect models.

Perfect recruit survival in all cold populations meant there was no data variation in the cold treatment, which precluded the use of mixed effects models. We tested whether hot populations had lower survival than cold populations in the F4 and F5 generations separately (pooling Selection). We then looked at whether survival in the hot populations was lower in the F5 generation than in the F4 (again, pooling Selection). This allowed us to investigate a temporal trend in hot recruit survival. We tested for differences in recruit survival between selection treatments (in hot populations only) in the F4 and F5 generations separately. Finally, pairwise comparisons of recruit survival were undertaken between all possible pairs of selection treatments in the F5 generation. The proportion of males as a function of total fish number was investigated independently for each fishery selection treatment where predictor variables included Temperature (two levels) and Generation (categorical two levels: F2 and F4). This allowed us to investigate trends in sex ratios within each fisheries selection treatment at finer scale

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than if we built the full model of all three factors. Models were assessed for overdispersion (66).

All mixed effect models contained similar random effects structures that reflected the underlying design of our experiment. The egg yolk size model included a Dish random effect nested within Population that was subsequently nested within Tank. The Dish random effect accounted for the multiple measures of individual yolk diameters taken from the same population in each generation. The Population and Tank random effects accounted for the nonindependence of observations within Populations through time, and across populations housed together. Mixed effects models were also fitted with a random intercept of Generation (categorical) to allow for random deviations in trait observations around a fixed linear generation trend of interest, except where specified below.

We modeled the egg yolk size, CV of egg yolk size, early survival and development rate response variables as a function of the fixed effects Temperature (two levels), Selection (three levels), and Generation (continuous), and their interactions. Egg yolk size, egg yolk size CV, development rate, and spawner size were modeled with a linear mixed effects model. Egg yolk size was log-transformed to deal with heteroscedasticity of residuals. We also explored the relationship between egg yolk size and spawner size and spawner size and fishing and warming treatments. Here, egg yolk size was fitted to spawner size (length or weight) and spawner size (length or weight) was also fitted to temperature treatment or selection treatment in separate mixed models. We fitted a generalized linear mixed-effect model to analyze early survival data, where an observation level random effect was fitted in place of all other random effects to account for model over-dispersion. In some instances, all eggs died within a dish (i.e., all eggs from a population within a generation). We fitted models including and excluding these mortality events and found they had no impact on the best fixed effect model structures. We therefore only report results that include these mortality events. Early survival and hatch rate models also included the number of eggs in each dish at the start of the incubation period as a fixed source of variation to improve estimation of our fixed terms of interest (Temperature, Selection, and Generation). We allowed this "Incubation density" factor to interact with temperature alone as we believe the relationship between hatch rate and incubation density may change with temperature due to acclimation or even evolution, but not with selection. For mixed effect and sex ratio analyses, we built a series of models (fit using maximum likelihood) for each response variable containing all combinations of fixed effects, including the null model, and ranked them using Akaike's information criterion corrected for sample size (AICc), and the difference between the best model (lowest AICc) and all other models (ΔAICc) (67). Optimal models were then reanalyzed using REML to produce unbiased parameter estimates (68).

Finally, we explored population's recovery rate in the first common garden generation (CG1). For this, we only investigated traits where we had already detected an effect of F1–F5 generation (i.e., early survival, hatch rate, and recruitment) in the previous analyses. For early survival and development rate analyses, we used the same model structure as applied to the selection phase data except that the Selection fixed effect was dropped, Generation was now included as an ordered categorical factor (with levels F5 and CG1), and the Generation and Population random effects were dropped as they only contained two levels. This left Tank as the single random effect in common garden hatch rate analysis and another observation level random effect for CG1 early survival. For recruit survival common garden analysis, we again applied a two-proportion z test and looked for higher survival in CG1 hot populations compared to F5 hot populations. In one instance, a sigmoid-hot population only produced a handful of unviable eggs during the spawning event in the F5 generation. This meant that not enough data were available to model reproductive and early life history responses from this point forward. We did, however, assign recruitment survival as zero in the F5 generation as we deemed the spawning failure a result of our treatments.

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