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Article

Observing food and density effects on the reproductive strategies of *Heterandria formosa*

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SUMMARY

Fishing and climate change have dramatically altered the structure of fish populations over the past century. Heavy fishing and changes in marine ecosystems affecting food availability are hypothesized to have altered not only abundances of fish species, but also important life history traits such as fecundity-body size relationships. Studying such hypotheses in the wild is difficult, so we use the live-bearing fish *Heterandria formosa* as a model for understanding such impacts more generally. We imposed different experimental harvest and feeding treatments over three generations. Different harvest treatments periodically removed subsets of the population either selectively with respect to body size–removing individuals with the longest body length–or non-selectively. Food treatments varied in whether fish were given constant or variable amounts of added food. To assess how females' length-specific fecundity and embryo size varied across treatments, for each generation, we subsampled females to measure their embryos. We hypothesized that selective harvest would increase females' length-specific fecundity. Our results showed fish from the selective harvest populations had higher length-specific fecundity compared to the non-selective harvests. However, these treatments also removed large fish, leading to populations of smaller fish with more embryos per unit mass. Therefore, harvests removing larger fish can alter life history, favoring greater reproduction at smaller sizes. While these changes may appear to bolster individual fitness, such changes may also detract from energy invested in growth and lead to unexpected shifts in individual fitness, population structure, and population viability.

INTRODUCTION

Fishing pressure has grown significantly over the past century leading to shifts in the productivity and dynamics of marine populations and ecosystems (1). In assessing such impacts on species and populations, much attention is paid to quantifying properties such as abundance, biomass, and population structure (2). However, effective conservation of fish populations also may require understanding how life-history traits, and traits in general ranging from physiological to behavioral processes which are not always well-studied in wild fish, respond to large-scale harvest (3). Specifically, many populations experiencing harvest (removal of some individuals from the population) by humans, especially harvest of large adults, have seen declines in traits such as size-at-age, or increases in size-specific fecundity, with an overall shift towards smaller but more fecund individuals (4, 5). Changes such as these in harvested populations are hypothesized to result from a combination harvest itself, and other ecological factors such as changes in food availability and competition (6). Harvest may directly affect such traits through evolutionary selective pressures. Such harvest-induced evolution is particularly thought to occur from size-selective harvest that selectively removes larger individuals (7). Although, harvest-induced evolution may also result from non-selective harvesting, which removes individuals across body sizes (7). Changes in fishing pressure have also coincided with shifts in environmental conditions, such as food availability and variability, that can also affect life history characteristics in both plastic and evolutionary ways (6, 8, 9). For example, reductions in food availability due to increased consumption of some food sources by newly-introduced species may both plastically reduce an individual fish's growth, and alter the selection and evolution its population experiences (6). Moreover, changes in population density that results from harvest or predation can affect within-species competition that in turn shapes life history traits (10). As a result, there is a need to disentangle how shifts in life history traits may be shaped by direct and indirect effects of harvest, as well as environmental effects.

Fishing pressure has the potential to drive observed changes in size structure, growth rates, fecundity, and maturity at age (7). These effects can arise from evolutionary pressures, which cause phenotypes in populations to change through shifts in the genetics of subsequent generations. Such changes may also result from plasticity, which is the non-heritable response of individuals' phenotypes to the environment (8). The effects of fishing pressure may depend on fishing intensity, size-selectivity, and any resulting changes

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in competition due to reductions in density (11). Specifically, fishing can directly affect life history by removing individuals that grow bigger and invest less in reproduction at smaller sizes (7). These selective pressures can favor individuals that allocate more energy to reproduction at smaller sizes (12). Yet such demographic shifts in fish populations, in both abundance and size structure, can also reduce competition within a population for food, space, mating opportunities, and other resources (4, 13, 14). Therefore, changes in mortality from predation or harvest may affect life history strategies through both evolutionary and plastic responses (14).

In wild populations, attributing shifts in traits to harvest or predation remains a distinct challenge because these processes occur in the presence of variable and changing external environments (6). Changes in productivity and availability of food is an important environmental factor that may affect populations and life histories (6, 8). For example, reduced food availability may negatively affect individual fecundity, survival of young, and/or size of offspring (15). Thus, trends in environmental variability may confound or mask any impacts of harvest or predation on life history traits. As a result, understanding how harvest may shape life history requires also evaluating how any shifts are affected by changes or variability in food productivity.

For many wild populations, attributing phenotypic shifts in life history to evolution versus plasticity, and environment versus harvest remains elusive or impossible (6, 10). In contrast, organisms with rapid generation times as well as known heritable and variable life history traits present excellent systems for testing how mortality (such as harvest) and environment might possibly shape life history in other systems (16). *Heterandria formosa* is a fast-growing, live-bearing fish species. These fish have a high level of superfetation, with females carrying several embryos of different developmental stages simultaneously (14). They are matrotrophic, providing ample nutrients to support the growth and development of their existing embryos (14). The number, size, and other characteristics of offspring may be influenced by environmental conditions experienced by the female (17). In wild north Florida populations of *H. formosa*, body of water type – pond versus spring – correlates across wild populations to characteristics of *H. formosa* embryos (10). Specifically, a female's offspring are generally smaller and more numerous in ponds, and larger and fewer in quantity in river springs (10). In addition to the patterns observed in the wild, *H. formosa* have been studied in controlled settings that further supported the effects of the maternal environment on female reproductive strategies such as the number and size of embryos she produces (17). Heritable differences in growth and size-specific fecundity between these north Florida populations have been demonstrated in laboratory common rearing experiments (15, 18). As a result, this species exhibits sufficient variation for testing how harvest and food may lead to plastic or evolutionary responses to such traits.

In this study, we used *H. formosa* to observe how different harvest and food conditions shape fecundity and size of embryos over multiple generations. Specifically, we tested whether different harvest size selectivity (removal of large individuals versus removal of individuals equally from all sizes) and variation in food availability (constant versus intermittent feeding) affected size-specific fecundity and size of offspring. We hypothesized that the removal of large individuals in contrast to removing random individuals would increase size-specific fecundity over time. We also hypothesized that variable food availability may reduce *H. formosa* fecundity and embryo growth. Our data support our first hypothesis that changes in population structure due to harvest increase fecundity across different female body lengths. Variable food did not have a consistent, significant negative effect on fecundity of females, and its relationship to fecundity differed strongly between seasons. The data we collected provides insight into how fish species may respond to fishing and environmental change.

RESULTS

In order to examine how size-specific reproductive investment in fish responds to both environmental changes and fishing, we dissected fish sampled from an ongoing multi-generation harvest experiment. Importantly, the responses to maternal conditions of the number and size of embryos these *H. formosa* fish carry are readily measured by dissection (17). This experiment exposed fish to different levels and types of harvest, and different levels of food variability. Different harvest treatments included no removals, removal of 60% of the population removing only the largest fish, or removal of a random sample of 60% of the fish at regular intervals. Within each harvest type, half of the population received constant food and the other half received variable food (**Figure 1**).

Our data show a hyperallometric relationship of fecundity to body length, and that the selective harvest treatment consistently increases fecundity across female body lengths. To compare treatment effects, we used a generalized linear model, with a Poisson likelihood and a log link, to estimate the scaling relationship between fecundity ($F_{i,j,t}$) and length ($L_{i,j,t}$) of the ith individual from the jth treatment at time t of each of the two timepoints included in the study. This generalized linear model fit a linear relationship between the natural log of female body length and the natural log of fecundity. Females at longer body lengths had disproportionately large numbers of embryos. Across all of these data, the rate at which fecundity increased with female body length - the slope of the linear relationship between log length and log fecundity - was estimated as β = 4.01 (95% credible interval = 3.39-4.64, using posterior quantiles). This means that fecundity did not scale isometrically with the cube of body length, and that proportionate female investment in fecundity instead increases with body length. The hyperallometric scaling relationship further differed between treatment groups (**Figure 2**). Across body lengths, the estimated fecundity for a female of any given body length was higher in the selective compared with the nonselective harvest treatment groups. Size-selective harvest fish had approximately 58% more embryos on average, after accounting for effects of body length, as compared to fish from non-selective harvest tanks (95% credible interval of for this effect of 0.20 to 0.74, or a 22% - 110% estimate increase given by exponentiating the estimated interval bounds to approximate the estimate percentage change). The probability estimated of a selective harvest effect larger than zero was 99.87%, and the estimated probability of a negative effect was 0.13%. This effect did not differ between timepoints analyzed (i.e., winter-spring of 2023 vs. late summer 2022; the model-given probability of a positive selective harvest timepoint interaction was 80.3%, not a significant effect). We describe here as 'significant'

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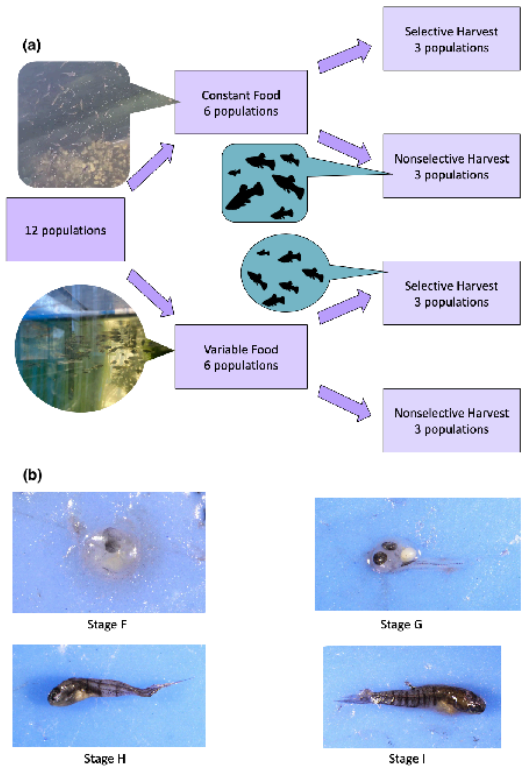


Figure 1. *H. formosa* treatment groups and embryonic developmental stages. (a) Graphic depicting treatment groups. 'Constant Food' refers to independent populations which received the same amount of food added each day, while 'Variable Food' tanks received the same amount of food on average but either extremely high or low daily amounts. In populations subject to 'Selective Harvest' the longest individuals in the population were periodically removed, while 'Nonselective Harvest' populations had individuals removed but equally from different body sizes. **(b)** Images showing developmental stages. These are how the embryos physically vary as they grow from the smallest and least developed stage, stage F, to being ready to be born. Photos and graphics by Lalima Sharan.

effects for which our 95% credible interval excludes zero and for which our model estimates probability of an effect in the estimated direction as above 97.5%.

The estimated effect of food variability in our data is dependent on the season in which the fish were sampled. The females we dissected from winter-spring 2023 timepoint had virtually the same average length-specific fecundity between the constant food and variable food treatment groups. However, during the late summer 2022 timepoint, constant food increased the length-specific fecundity of females relative to those fish from the variable food treatment group, with our statistical model showing a significant interaction effect between time period and the food treatment. Variable food compared to constant food is estimated to have reduced the average total number of embryos (for any given female body length) on the log scale by -0.29 (a reduction by about -25%, the 95% quantiles credible interval ranging from -44% to +1%) for fish of any given length during summer of 2022, with a model-given probability of an effect below zero of 97.02%. During spring of 2023, variable food compared to constant food is estimated to have increased the average total number of embryos (for any given female body length) on the log scale by 0.03 (an increase by about 3%, the 95% quantiles credible interval ranging from about -23% to +39%) for fish of any given length. Therefore, the size of the estimated effect of food on the number of embryos changes from one timepoint to the next. The estimated effect of food on fecundity, on the log scale, is -0.29 in summer, but it is about +0.03 in winter/spring. This change through time is why we observe a relatively large estimated timepoint-food treatment interaction effect. The model-estimated value for an interaction effect between the variable food treatment and timepoint was 0.32 (95% credible interval ranging from 0.04 - 0.61). The model-given probability of this interaction effect being greater than zero is 98.76%, a significant interaction effect. Food treatment does not appear to vary in its effects based on the ongoing harvest treatment. A model fit to estimate an interaction between the effects of harvest treatment and food treatment did not find a significant interaction (credible interval for the interaction effect ranging from -28% change to +112% change).

This difference between timepoints aligns with how the fish populations differed across timepoints in their total amount of feeding activity, as measured in video recordings. Fish filmed in summer of 2022 finished their food much faster and therefore ate more than those in the winter/spring. In the spring, out of 13 videos taken during feeding across the 36 tanks in different treatment groups, we never observed fish finishing their flake food in under 10 minutes. In contrast, in the summer, 36% of trials (N= 17) resulted in fish finishing their flake food 10 minutes after it was added, with 24% of tanks finishing it in under 5 minutes. This resulted in a significant difference between periods with a reduction in log odds of finishing food of -10 in winter (95% credible interval of -34.2 to -1.8) under a binomial generalized linear mixed model.

In contrast to shifts in fecundity, the mean maximum embryo length for each individual was generally consistent across harvest treatments, female size, and time period (**Figure 3**). Embryo length increased allometrically with female body size. The scaling exponent of maximum embryo length within a female with female body length was 0.38 (95% credible interval 0.08 to 0.68), indicating a decelerating relationship between maximum embryo length within a female and female body length. There was a small effect of variable food resources treatment on mean maximum embryo length with a reduction of -0.11 on the log scale (~10% reduction in maximum embryo length within a female due to variable resources). However, the 95% credible interval did include zero (95% credible interval of -0.23 to 0.01, 96.8% estimated probability of a positive effect using bayestestR). There was no difference between harvest treatments. The estimated probability of an effect greater than 0 of selective harvest on embryo maximum length was about 51.8%.

DISCUSSION

Size selective harvests can directly affect populations by truncating size structure and reducing population density (4). Such changes are thought to present both ecological and evolutionary feedbacks that shape life history by changing selective pressures and reducing competition (11). As a result, many theories suggest that investing comparatively less in growth and more in reproduction in these scenarios confers a fitness advantage, leading to a steeper fecundity-at-length relationship and slower growth rate (12). In this study we show a substantial shift in a key life history trait associated with experimental, size selective harvest over 3-4 generations in

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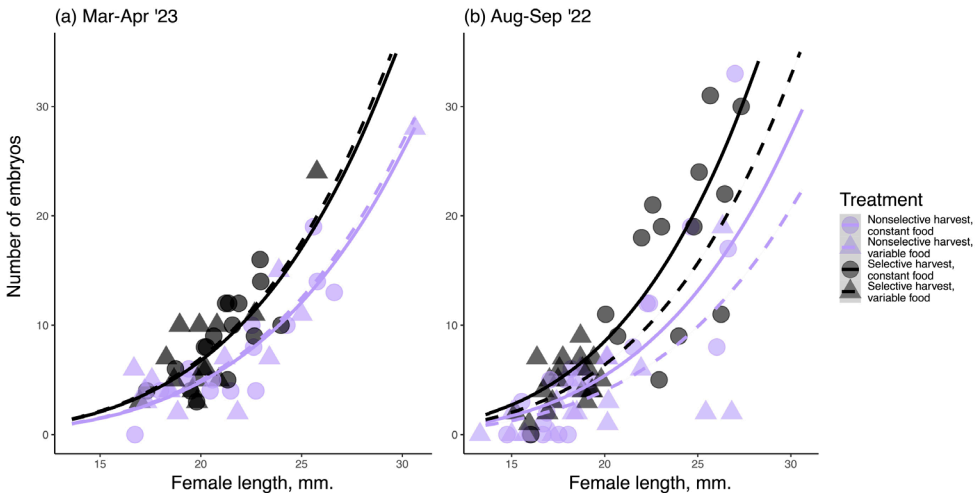


Figure 2. Comparison of the effects of harvest selection and feeding schedule on the number of *H. formosa* embryos per female at different timepoints. Effect of the different treatments (constant or variable food and selective or non-size-selective harvest) on the number of embryos for any given female length in **(a)** March–April (2023) or **(b)** August–September (2022). Data were collected from 57 (for Mar–Apr) or 73 female fish (Aug–Sep), across 12 independent groups. Fitted lines are from a Poisson log-log generalized linear model.

live-bearing fish. Specifically, fecundity-at-length increases under size selective versus non-selective harvest.

Increased length-specific fecundity at the individual level in size-selective tanks did not necessarily lead to more total offspring produced by entire populations. Such changes in size specific fecundity are also associated with changes in the population as a whole, such as shifts in the size distribution. Specifically, most fish were relatively small in the size-selective harvest tanks. Since body length is one of the most important determinants of total fecundity in *H. formosa*, the total number of new offspring in size-selective harvest tanks may still be lower than that in non-selective harvest tanks, despite increased length-specific fecundity due to size-selective harvest (17). Whether increased size-specific fecundity leads to more or fewer new offspring produced by the population as a whole also depends on whether there is a tradeoff between increasing reproduction at small body lengths, and lifetime reproduction as well as survival of young (12). Additional feedback between these population characteristics and mean individual body size may further complicate population-level outcomes (11). For example, increased population-wide reproduction may lead to increased population size depending on the competitive environment that new offspring face (19). More reproduction could reduce population size if there is too much competition for these offspring, resulting in fewer offspring that make it past the egg and larval stages (19). Additionally, a variety of traits beyond life history (e.g., disease tolerance, physiological rates) may fluctuate in populations experiencing ecological and evolutionary changes due to harvest (20). Importantly, many of these changes may indirectly affect individual and population-level fecundity.

The observed increase in size-specific fecundity in response to size-selective harvest may be due to at least two non-mutually exclusive processes. First, the shift may result from an evolutionary response. Such an outcome has been

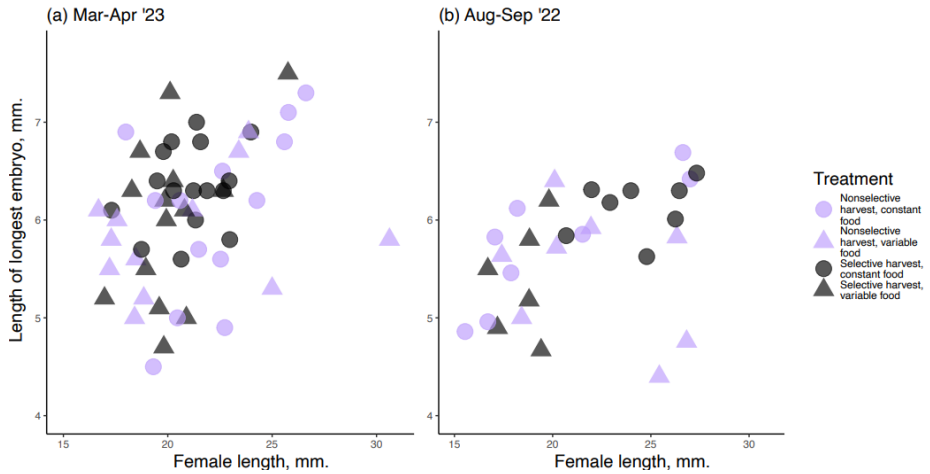


Figure 3. Comparison of effects of harvest selection and feeding schedule on *H. formosa* embryo length, the maximum length of the embryos within a female, at different timepoints. Effect of the different treatments (constant or variable food and selective or non-size-selective harvest) on the lengths of the longest embryos for any given female length in **(a)** March–April (2023) or **(b)** August–September (2022). Data collected from 57 (Mar–Apr) or 32 female fish (Aug–Sep), across 12 independent groups.

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shown in a meta-analysis of various animal species in which life-history traits were shown to rapidly shift following increases in predation favoring large adults (5). Alternatively, shifts in size-specific fecundity may also result from competitive release. Reductions in the density of large individuals may lead to higher overall resource availability for small animals, resulting in more energy available to smaller individuals for egg production. Specifically, bigger animals may be better competitors, have higher overall consumptive demand, and have substantially higher reproductive rates; these factors are likely true in wild *H. formosa* populations (10). Thus, food availability for smaller individuals may increase because of less competitive exclusion, or through increases in food availability due to lower demand by fewer bigger individuals or lower overall recruitment. *H. formosa* are known to increase fecundity in response to reductions in density and increase in per capita food (17). Importantly, these evolutionary and plastic processes may occur simultaneously. Determining whether evolutionary and/or plastic processes are at play could be tested using common-garden rearing or genetic sequencing to distinguish genetic and non-genetic effects in these harvest-based results (5).

In contrast to harvest, there was no significant main effect of food variability on fecundity. This is consistent with findings that *H. formosa* fecundity may be more affected by population density than food (17). There was, however, a significant positive interaction between food variability and timepoint on the number of embryos. While the embryos from the March-April group had little variation in number of embryos with food treatment, data from a group harvested in the previous summer showed a significantly larger difference. The March-April harvest group may have exhibited no differences in size-specific fecundity with food treatment because the fish ate less in the winter. As temperature decreases, so does the rate of metabolism (21). As a result, fewer calories are expended and less food is needed to maintain energy. Moreover, the birth rate of *H. formosa* declines in winter and thus overall population densities decrease. This is also observed in the wild for this species (10). Therefore, food limitation may have less importance in colder temperatures than in warmer seasons. This hypothesis is consistent with findings in most other ectotherm systems including other freshwater fish, in which metabolic rate is reduced in low temperatures (21-23).
In contrast to embryo number, embryo size did not vary significantly throughout the experiment, or among treatments. This result suggests that population density is more likely to affect the number of embryos, rather than embryo size. These findings are consistent with previous work on *H. formosa* where density affects fecundity but not embryo size (17). Other fish systems have also shown an increase in larval quantity but not size or quality due to density manipulation (24). Additionally, higher statistical power from collecting more data points than analyzed in this study might allow sufficient precision to identify consistent but small effects on individual embryo length. Fishing regimes in real wild harvested fish populations introduce many mortality patterns different from just those two simulated in this study, and different patterns could have produced different results in this species (7).

Overall, changes in harvest patterns and food variability had significant effects on size-specific fecundity, a key life history trait. We found that removing large fish from a population results in substantial increases in size-specific fecundity. Importantly, these shifts do not necessarily compensate for the higher fecundity of larger individuals. Large fish that are more common in the non-selective treatments than in the size-selective treatments still have disproportionately higher fecundity than smaller fish due to the hyperallometry of fecundity, with higher mass-specific fecundity than expected as fish increase in size. These effects persist when food level is variable, with food variability not having the detectable significant effects on fecundity exerted by the harvest treatment. These results present important experimental outcomes that may inform expectations for wild, harvested species where, for many taxa, experiments remain infeasible. Thus, these results lend insight into how harvest approaches and environment can directly affect key life history characteristics.

MATERIALS AND METHODS

Experimental populations

Fish used in this experiment (Florida State University animal care protocol TR202300000001) were bred from a genetically diverse mixed stock descended from fish collected from wild populations. Fish were collected from two Florida Panhandle sites, Trout Pond and Wacissa River. The life history and reproductive biology (including genetic and plastic differences) of fish at these sites have been well-described by previous research (15). About 690 pure-bred fish were initially collected in multiple trips in July 2020, November 2020, and March 2021, using long dipnets. About 140 fish were collected in July 2020, 300 were collected in November 2020, and 250 were collected in March 2021. Fish were initially stored in outdoor 150-gallon cattle tanks. For the duration of this work they were moved to the FSU Biology Mission Rd. greenhouse in Tallahassee, in identical 29-gallon tanks. This greenhouse space has natural variation in light and seasonal variation in water temperature ranging from 15-35°C. However, radiators in the greenhouse prevented any near-freezing temperatures in the winter, and a swamp chiller system turned on at air temperatures above about 32°C.

To create the mixed populations for the experiment we generated a pool of F1 and F2 individuals by allowing adults from both populations to breed. Approximately 400 remaining fish from 2020 were bred together (by placing them in tanks with females of one ancestral population and males of another) beginning in late February 2021 and supplemented with 250 fish collected in March 2021. About 170 of these total 690 fish were from Trout Pond. Most of the breeding tanks were Trout Pond males x Wacissa females; fish were harder to find at Trout Pond, and females from the Wacissa River population were better at sustaining embryos sired by Trout Pond male sperm than the reverse, as previously reported (18). The F1 fish were bred together to make F2s and then the F1 and F2 populations were mixed to start experimental populations in November 2021. Each independent population began with 78 fish, a mixture of F1s and F2s with the same distribution of body lengths. For each length class, the fish that were chosen for a particular population were randomly drawn from those available of that length.

Treatments and harvesting

After independent replicate populations were bred and assembled, we crossed two continuously applied levels of variability in food with three levels of a discretely applied

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harvest treatment. Populations were randomly assigned to treatments, with treatment groups arranged in a spatially blocked design so that each of the six possible treatment combinations were spread throughout the space. Fish were removed from some populations approximately every three months to simulate harvest. Populations were not harvested at all (not discussed in this paper), had all individuals longer than the population's 40th percentile in body length removed (size-selective), or had 60% of individuals from each length group removed (non-selective). For the latter two groups, the female fish removed and euthanized were dissected to measure the embryos they were carrying when they were alive. Each independent population was kept in a pair of 29-gallon tanks and mixed evenly between this pair of tanks when fish were taken out, measured, and sampled at the harvest intervals. This gave the populations enough space for many individuals, while maintaining the genetic pool of each population.

Each period of harvesting individuals from tanks took at least a month, during which time the temperature varied. To address this, harvesting was consistently done in the same order by spatial block, avoiding confounding treatment combinations with the harvesting and euthanizing order for each timepoint. Nevertheless, we did include temperature in some exploratory statistical model fitting to account for variation from day to day in temperature within the timepoint for that harvest. This variation only affected the number of embryos by about 1% per degree Celsius, and did not qualitatively change other estimated effects.

To measure within-day consistency in temperature between different tanks in our experimental space, we recorded temperatures daily with glass thermometers placed in six different tanks, choosing to deploy the thermometers in tanks distributed widely apart from each other to best measure the heterogeneity in temperature between tanks at the same day and time. Over 165 days of measuring the same six pairs of tanks daily, the average range in temperature between the warmest and coolest tank at any given time of day was 1.9°C, smaller than the typical shift in temperature over the course of the day (which was often 2-3°C or more), or the 15-20°C variation from winter to summer. Different parts within the greenhouse space were likely affected differently by temperature at different times of the day (e.g., sunlight was more direct on the east side of the room during the morning). Within each of the spatial blocks, the average difference in temperature at the same day and time between two tanks was less than 0.6°C.

Within each level of the harvest treatment, half of the populations received constant food (finely crushed TetraMin® flake food) and the other half received variable food (**Figure 1**). The variable food treatment was on average the same amount of food as the constant food treatment over the duration of the experiment but alternated between days with either 20mg or 1,180mg food per day, in contrast to the constant food populations which received 600mg per day. Food levels were set by determining what levels per capita would be appropriate for typical growth by *H. formosa* and the initial population of 78 fish per experimental replicate (17). Food amounts were unchanged throughout the experiment so that tanks reached population densities that promote competition for food. For each two week period of the experiment, the 7 days out of the upcoming 14 days that would be "low-food" days were randomly chosen using the sample() function in R, so populations rarely went more than 3 days on minimal food. Custom cut acrylic measuring spoons were used for measuring volumes of fine-crushed flake food corresponding to intended weights. The resource treatment began when the populations were initialized and continued throughout the entire experiment. Feeding was done at the same time each day for each tank, usually between 10am-2pm, with some variation (though always during daylight hours).

To keep water chemistry consistent and safe for fish, and to remove as much algae as possible (which could serve as an additional food source), we scraped the walls and siphoned the floors of the tanks and changed ~50% of the water in each of the tanks roughly every 2-3 weeks. We also tested for water quality parameters on a similar schedule. Parameters tested for regularly were nitrates, nitrites, pH, ammonia, and chlorine compounds, which were all stable at safe levels in all the tanks throughout the experiments. Further methods to prevent algal growth included: shading on the greenhouse roof, white corrugated sheet plastic lids covering the tanks (although not preventing air entry into the tanks), *Elodea* plants from Carolina Biological to compete with algae while providing (non-edible) habitat to fish, and sponge filters (brand Aquacity) to provide aeration as well as some water circulation and removal of particulates. Partway through the summer (July-August) of 2022, we introduced more and improved evenness of shading over all the tanks. In addition to plastic tarp already hung, more plastic shading and umbrella material were hung to ensure all tanks were blocked from direct sunlight for most of the day. This helped to reduce differences between tanks in amounts of algae growth.

To score overall feeding rate, we conducted video analysis of feeding events in 10-minute trials to assess whether or not fish finished their flake food within a certain standardized period of time. Whether fish finished their food in this time period acted as a rough estimate of total food demand in the tank. Overhead videos of fish feeding in the tanks after addition of flake food were taken. These videos were taken of most tanks in Feb-Apr 2023 and Jun-Aug 2023. The data includes videos taken from at least two independent populations from all six treatment combinations for each of the timepoints. Observers used iPhone cameras resting on an observation platform above the tank with the camera pointed down at the tank. When the video was taken for a tank, it had not been fed since its prescribed feeding the previous day. The platform was left in place on the tank for five minutes prior to videos being recorded to allow fish to acclimate to its presence. Recordings were initiated, food was added thereafter, and videos were recorded for 10 minutes. Observers left the vicinity of the tank during recordings to minimize disruptions to the fish. To score whether fish finished their food by the time allotted, we observed whether or not there were any pieces of flake food still visible floating on the water's surface.

Data collection and analysis

Harvested fish were frozen and retained for collection of data on traits, such as fecundity, that could only be obtained from dissection. Individuals were measured by length and then at least four females from each treatment group were selected to be dissected. We chose fish across a range of body lengths, ensuring that size distributions of analyzed fish overlapped between tanks, to be able to assess differences

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between treatment groups in females of the same body length. This was necessary as long body lengths were less common in the populations being selectively harvested for long lengths. This ensured, for instance, that each independent population had multiple females below and above 20mm body length in these data. The selected female fish were dissected and their embryos were counted, measured using a Nikon SMZ1270 microscope, and classified by developmental stage. We counted all embryos which were of developmental Stage 7 or higher using a previously developed system of developmental stages for *H. formosa* embryos (embryos smaller and less developed than Stage 7 were not within our technical capabilities to consistently find, measure and describe) (25). We used generalized linear models to describe the relationships between our measured trait data and the conditions in the tanks, and to estimate the probability that these experimental conditions affect the traits we measured. For data on total numbers of embryos recorded, we used a generalized linear model fit assuming that the data are distributed according to a Poisson distribution, as is appropriate for integer count data. To compare treatment effects, we estimated the relationship between fecundity ($F_{i,j,t}$) and length ($L_{i,j,t}$) of the ith individual from the jth treatment at time t:

$$(F_{i,j,t}) = \alpha_{j,t} L_{i,j,t}^{\beta} \qquad \textbf{(Equation 1)}$$

where the parameter ($\alpha_{j,t}$) is the scaling constant for each treatment at each timepoint and β is the common exponent. Differences in the estimated scaling factor ($\alpha_{j,t}$) for the relationship of the number of embryos to length between treatment groups corresponded to estimated differences between treatment groups in length-specific fecundity. For data on maximum embryo length for each dissected female, we assumed a gamma distribution of the outcome variable. We used a binomial distribution of the outcome with a logit link function for whether or not a tank finished its food in the feeding analysis. We used random effects to account for the non-independence of fish from the same experimental replicate. All models were fit in R version 4.3.1, package brms, using package bayestestR for probabilities of directions of effects (26, 27).

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