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Size-dependent resource allocation to reproduction in Japanese anchovies (*Engraulis japonicus*)

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Size-related changes in reproductive potential are essential for understanding population dynamics and life history strategies; however, the mechanisms driving variation between individuals and sexes remain unclear. Using a diet-switch experiment and carbon and nitrogen stable isotope ratios (SIRs) as quantitative indicators, we investigated how large (parents) and small (offspring) spawning male and female Japanese anchovies (*Engraulis japonicus*) allocate energy to reproduction. Large individuals of both sexes exhibited higher energy investment in gamete production than small individuals, reflecting a trade-off between growth and reproduction. The turnover rates of SIRs after a diet switch suggest that anchovies predominantly rely on income breeding. However, significant differences in turnover rates between large and small individuals indicate that large fish can also utilize facultative capital resources for gamete production. Notably, males exhibited slower isotopic turnover than females, partly due to the influence of sperm storage. These findings demonstrate that spawning individuals display temporal diversity in resource use for reproduction, with large individuals showing greater flexibility in energy allocation strategies. This study advances our understanding of size- and sex-dependent resource allocation in Japanese anchovy and contributes to a broader understanding of reproductive strategies in small pelagic fishes.

Life history theory posits that organisms allocate their reproductive output and resources among growth, reproduction, and self-maintenance to maximize lifetime reproductive success[1,2]. Because organisms typically prioritize self-maintenance, energy allocation trade-offs often arise between growth and reproduction[3]. Once an animal reaches sexual maturation (puberty), the resources previously allocated to growth are being reallocated to reproduction. Thus, size-dependent resource allocation to reproductive output can be determined throughout the reproductive lifetime of an animal. Although theoretical growth models often assume that reproduction is proportional to body size[4,5], in several marine and terrestrial organisms, larger individuals allocate disproportionately more energy to egg production than smaller individuals by increasing relative fecundity (total number of eggs produced per body weight) and egg size[6,7]. In aquatic organisms, egg size is associated with several fundamental and adaptive traits of offspring[8], and the larvae that hatched from larger eggs generally grow larger and faster than those that hatched from smaller eggs. Therefore, these initial egg modifications may affect offspring growth, survival, and reproductive success. The effects of maternal attributes on offspring viability have been recognized as important components of population productivity and resilience in marine fish[9–11]. Although size-related resource allocation to reproduction may be similar between male and female fish, allocation to sperm production could change with mating systems or behaviors; male reproductive success generally increases with the number of sperm released during fertilization, while alternative mating tactics such as sneaker or satellite behaviors could enhance their chances of reproductive success[12–15]. Thus, resource allocation to reproductive output may differ between male and female fish; however, research on the mechanisms behind such a difference in resource allocation between male and female fish is limited.

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Two types of energy allocation strategies influence the selection of life history traits and the sensitivity of reproductive output to local environmental conditions[16–19]. For income breeders, reproductive output should be closely linked to current resource availability, whereas for capital breeders, energy acquisition should be temporally separated from investment in reproduction. Both types of energy allocation strategies have associated costs and benefits; long-term energy storage may allow capital breeders to cope with unpredictable environments or low food availability, whereas income breeders may increase their reproductive output more rapidly in response to an increase in local food supply. Income breeding may be ideal for marine organisms with a small body size but high fecundity through multiple-batch spawning, as income breeding can mediate the energetic paradox between low body reserves and high reproductive output[20,21]. However, increasing evidence suggests that income and capital breeding represent idealized endpoints of a continuum of reproductive strategies[22,23]. Most individuals do not rely solely on one of these strategies, but rather use a combination, depending on environmental conditions and physiological state[20–23]. In response to intrinsic and extrinsic factors (e.g., decreasing food availability), spawning females can adjust their macronutrient routing to fuel reproduction and reproductive output to enhance both reproductive success and maternal survival[24,25]. Functionally, body size and condition are key phenotypic traits of spawning organisms that buffer them from challenges through flexible energy management.

Traditional methods for studying capital versus income breeding rely on correlating ovarian development with macronutrient loss in spawning females[20,26]. For example, macronutrient levels in capital breeders significantly decline due to the depletion of energy reserves, whereas those in income breeders remain unchanged. However, maternal conditions could also decline due to inadequate nutrition; hence, a decrease in mass may result from income breeding. Bulk tissue isotopes provide a quantitative means of directly tracing the relative contributions of endogenous and exogenous nutrient sources to reproduction[27]. Techniques using stable isotopic tracers have allowed researchers to monitor how female fish use and allocate endogenous and exogenous resources to reproduction, thereby distinguishing between income and capital breeders[22,25,28,29]. In contrast, there has been little research on how male fish allocate resources and where they fall on the capital–income breeding continuum using such techniques[19].

The purpose of this study was to elucidate size-related energy allocation between body growth and reproductive output in male and female Japanese anchovies (*Engraulis japonicus*) under captive conditions. Anchovies (Engraulidae) are small schooling fish distributed in the oceans worldwide[30]. It is a typical forage and commercially important fish, and its biomass changes over a multidecadal cycle[30,31]. Similar to other anchovies, the Japanese anchovy (hereafter called anchovy) is a short-lived fish with a lifespan of 1–3 years and exhibits multiple-batch spawning over a prolonged season[32–34]. Female anchovies are income breeders because they largely depend on the energy acquired for egg production during the ongoing spawning season[21]. However, how body size influences energy allocation in adult female anchovies remains poorly understood. On the other hand, no information is currently available on how male anchovies allocate energy to sperm production and on whether individuals can adjust their macronutrient routing to fuel reproduction during their reproductive lifetimes. This study addresses these gaps by investigating size- and sex- specific isotopic turnover in muscle and gonadal tissues, revealing trade-offs between growth and reproduction and providing novel insights into resource allocation strategies in Japanese anchovy. Here, the growth and reproductive traits of parents (large) and offspring (small) were compared under identical environmental conditions using a diet-switching approach with two different values of stable isotope ratios (SIRs, Table 1) as quantitative indicators[21]. Diets with high SIRs were initially provided for several months and then switched to diets with low SIRs to determine the SIR turnover rate in tissues. Body size, reproductive traits, and SIRs of muscles and gonads after the diet switch were monitored to determine how income resources were allocated to growth and reproduction. The main objectives of this study were to: (1) compare energy allocation to gamete production between large and small individuals of both sexes; (2) determine any differences in the SIR turnover rates of the gonads between large and small individuals and between sexes.

Abbreviation	Definition
BF	Batch fecundity
BW	Body weight
DEW	Dried egg weight
DI	Degree of dependence on income resources
k	Turnover rate calculated by Eqs. (5) and (8)
SIR	Stable isotope ratio
SL	Standard length
SW	Somatic weight
$T_{50\%}$	Time (days) required for isotopic value to reach 50% of its equilibrium
TC	Total carbon content
TN	Total nitrogen content
TW	Testicular weight

Table 1. List of abbreviations used in this study.

Results

Growth traits

The standard length (SL, Table 1) and somatic weight (SW) of parents showed no significant changes over the sampling period ($P=0.40$ for SL and $P=0.82$ for SW), indicating minimal skeletal and somatic growth in large individuals during the experiment (Fig. 1; Supplementary Table S1). In contrast, the SL and SW of offspring significantly increased ($P<0.001$), with SL and SW growing by approximately 26% and 115%, respectively, by the end of the experiment. Additionally, the SL and SW of female parents and offspring were significantly higher than those of males ($P<0.001$ for parents SL and $P<0.01$ for offspring SL and SW). The $\delta^{15}N$ and $\delta^{13}C$ of the muscles of the parents and offspring significantly decreased ($P<0.001$, Fig. 2, Supplementary Table S2); however, the rate of isotopic turnover (slopes) in offspring muscles was approximately 2.7 or 3.0 times higher than that of the parents. Significant sex-based differences in $\delta^{13}C$ were found in offspring ($P<0.01$) while differences in $\delta^{15}N$ were observed in parents ($P<0.001$).

Reproductive traits

There was a significant difference in the relationship between testicular weight (TW) and SW between fathers and sons ($P<0.001$, Fig. 3a, Supplementary Table S3); within the same SW range (11–15 g) although there was no significant effect of SW on TW ($P=0.15$), the expected TW of small males (TW = 0.74 g) was approximately 56% of the TW of large males (TW = 1.33 g, $P<0.01$). Similarly, there was a significant difference in the relationship between batch fecundity (BF) and somatic weight (SW) between mothers and daughters ($P<0.001$, Fig. 3b); within the same SW range (13–21 g) the expected BF of small females was approximately 72% of the BF of large females with the same SW (e.g. fish with 15 g SW = 6522 oocytes for daughter and 9092 oocytes for mother, $P<0.001$). Spawning frequency did not differ significantly between the mothers and daughters ($P=0.73$, Fig. 4a, Supplementary Table S4). However, the dried egg weight (DEW) of the daughters was significantly lower than those of their mothers ($P<0.01$, Fig. 4b). There was a significant difference in reproductive effort per spawning between large and small females ($P<0.001$, Fig. 4c), as the expected reproductive effort of the small females was approximately 67% of that of the large ones.

In males, the $\delta^{13}C$ and $\delta^{15}N$ of the testes significantly decreased throughout the experiment; however, there was a significant difference in the plateau of $\delta^{13}C$ (-20.27 for fathers and −20.87 for sons, $P<0.05$) or turnover rate (k) of $\delta^{15}N$ (0.07 for fathers and 0.31 for sons, $P<0.05$) between the fathers and sons (Fig. 5a, b, Supplementary Table S5). The estimated half time required for its initial difference from equilibrium ($T_{50\%}$) of $\delta^{13}C$ and $\delta^{15}N$ of the testes of the sons were markedly lower than those of the fathers ($\delta^{13}C$ = 3.16 days for son and 4.35 days for father, $\delta^{15}N$ = 2.21 days and 10.16 days). The $\delta^{13}C$ and $\delta^{15}N$ of the ovaries of the mothers and daughters sharply decreased at the beginning of the experiment, followed by a rapid stabilization and plateauing

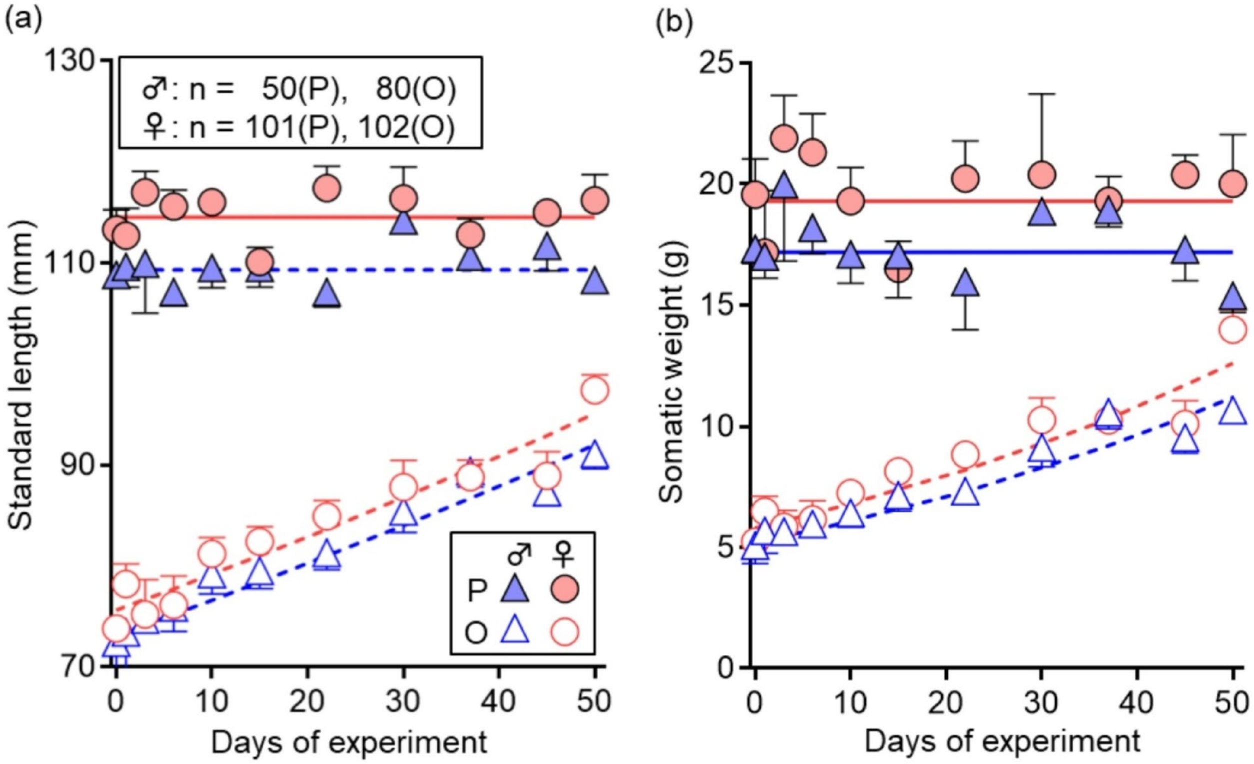

Fig. 1. Changes in standard length ((**a**), mm) and somatic weight ((**b**), g) of male and female parents and offspring Japanese anchovies during the experiment. Data are presented as mean ± standard error (SE). A linear mixed effect model was fitted to the observed data.

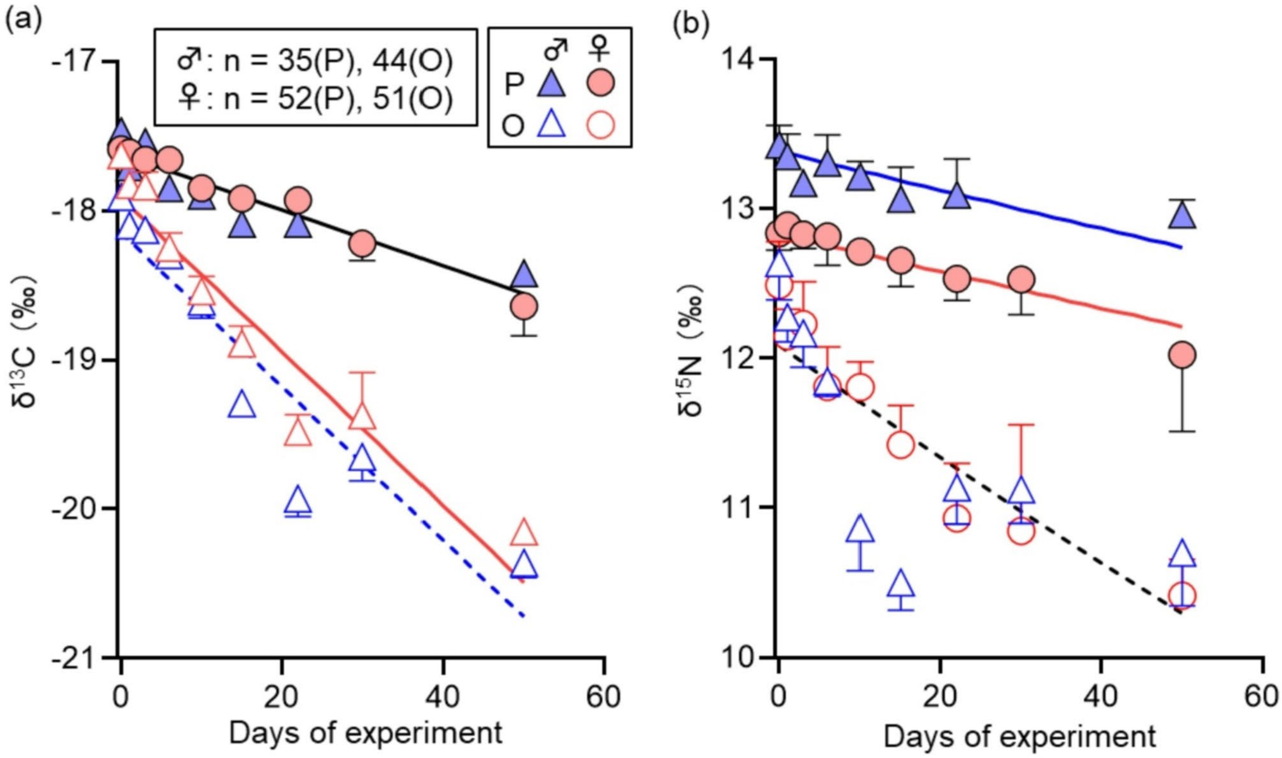

Fig. 2. Changes in $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values (‰) of muscle tissues in male and female parents (P) and offspring (O) of Japanese anchovies during the experiment (days after prey change). Data are presented as mean ± standard error (SE). A linear mixed effect model was fitted to the observed data.

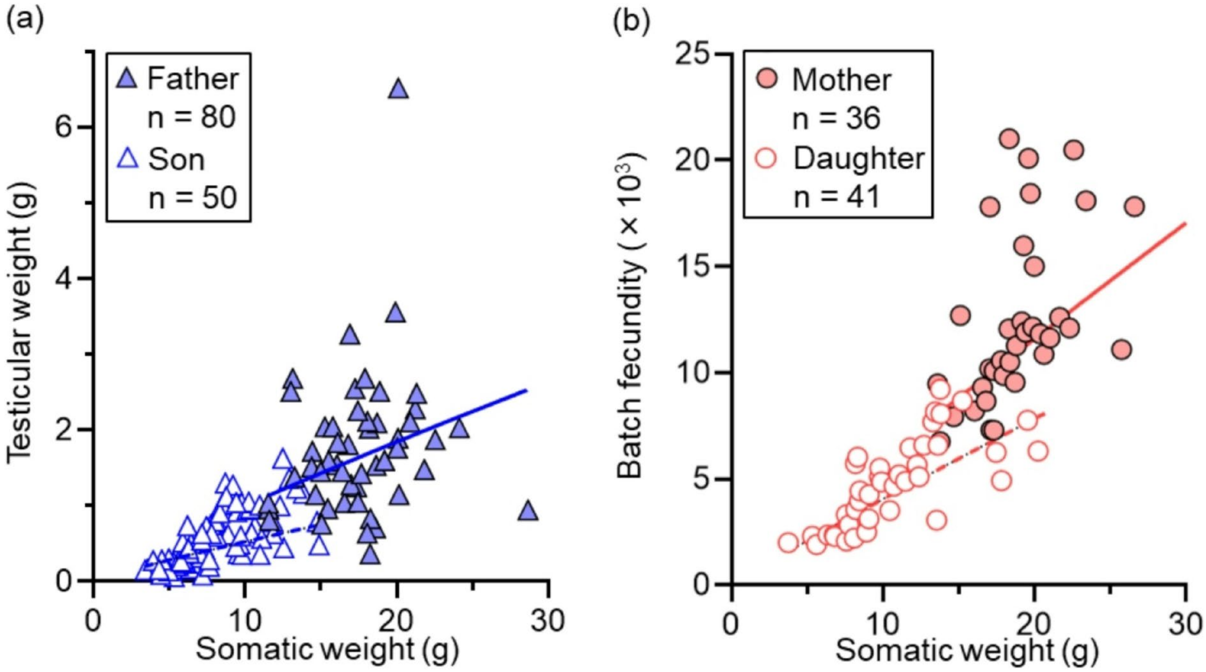

Fig. 3. Relationships between testicular weight (g) and somatic weight (g) of fathers and sons (**a**) and between batch fecundity (g) and somatic weight of mothers and daughters (**b**) in anchovies. A linear mixed effect model was fitted to the observed data.

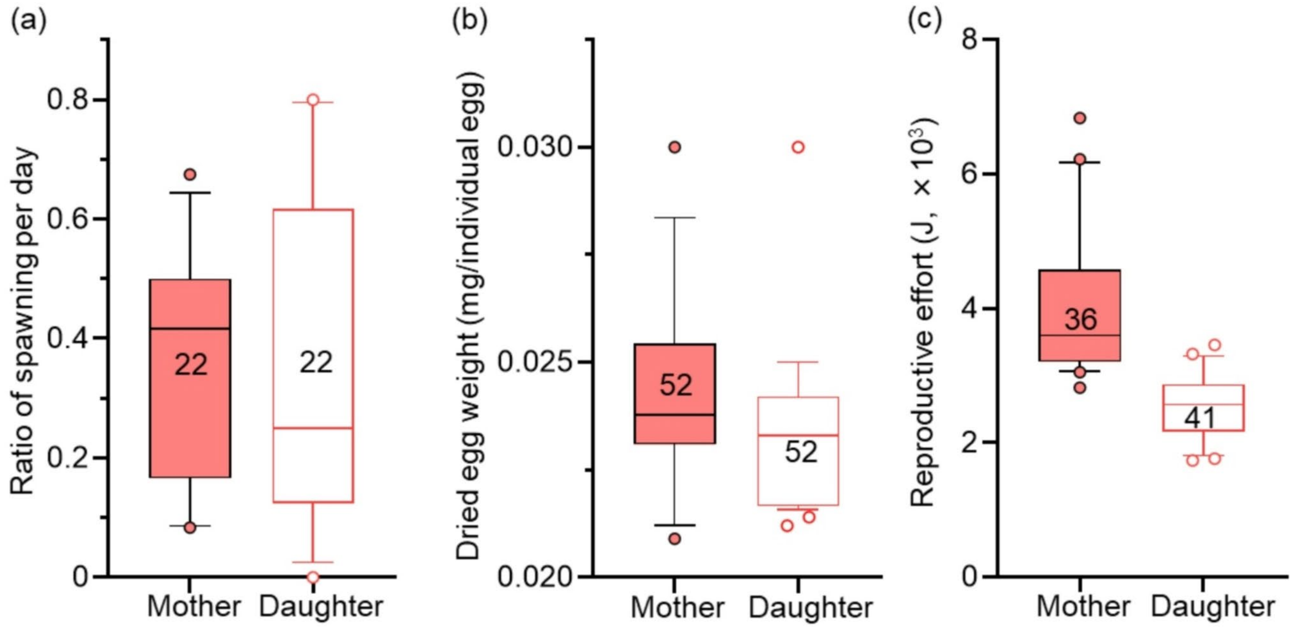

Fig. 4. Boxplots showing spawning frequency ((**a**), ratio of spawning per day), dried egg weight ((**b**), mg/individual egg) and reproductive effort ((**c**), J) of mother and daughter anchovies. The central line in each box represents the median, while the box edges represent the interquartile range. Whiskers represent 10th percentile and up to the 90th and individual points (circles) indicate outliers. Numerals indicated the number of sampling trials (**a**,**b**) and fish examined (**c**).

(Fig. 5c, d); however, there were significant differences in the k of $\delta^{13}C$ and $\delta^{15}N$ between the mothers ($\delta^{13}C$ = 0.21 and $\delta^{15}N$ = 0.22) and daughters ($\delta^{13}C$ = 0.36 and $\delta^{15}N$ = 0.60, $P < 0.01$). The estimated $T_{50\%}$ of $\delta^{13}C$ and $\delta^{15}N$ were approximately 3.3 days ($\delta^{13}C$ = 3.34 days and $\delta^{15}N$ = 3.21 days) for the mothers and 1.6 days ($\delta^{13}C$ = 1.95 days and $\delta^{15}N$ = 1.15 days) for the daughters.

Comparison of the degree of dependence on income resources between parents and offspring

For the muscles, the degree of dependence on income resources (DI) of the parents and offspring increased during the experiment; however, the slope of the DI increase in offspring was approximately 2.8 times steeper than that of their parents (Fig. 6a, Supplementary Table S6), reflecting a faster turnover of income resources in smaller individuals. At the end of the experiment, the DI of the offspring was approximately 0.71, whereas that of the parents was approximately 0.25.

For the gonads, the DIs of the parents and offspring of both sexes increased asymptotically and converged around 1.0 by the end of the experiment (Fig. 6b, Supplementary Table S6). Offspring exhibited significantly higher turnover rates (k values) compared to parents in both sexes. Furthermore, females showed large k values than males across both size classes. The estimated $T_{50\%}$ of isotopic incorporation was approximately 2.6 days for the sons, 7.1 days for the fathers, 1.5 days for the daughters, and 3.3 days for the mothers.

Discussion

This study demonstrated that body size and sex significantly influence energy allocation strategies in multiple-batch-spawning Japanese anchovies (*Engraulis japonicus*), revealing distinct trade-offs between growth and reproduction. Small individuals prioritized somatic growth, while large individuals allocated more resources to reproductive output, reflecting size-dependent reproductive tactics. Although anchovies primarily rely on income resources for reproduction[21], the dynamics of stable isotope ratios (SIRs, Table 1) in gonads indicate temporal flexibility in resource use between individuals of different sizes and sexes. These findings are consistent with the continuum model of reproductive strategies, where income and capital breeding coexist as adaptable tactics[18–25]. By integrating isotopic analysis to quantify resource allocation, this study provides a deeper understanding of the mechanisms driving reproductive strategies in small pelagic fish and highlights the importance of size and sex in shaping reproductive outcomes.

Our findings showed that the SL and SW of the parents remained constant during the experiment, whereas those of their offspring rapidly increased. By the end of the diet-switch experiment, the DI of the muscle tissues of the offspring increased approximately 2.8 times more than that of the parents. Isotopic turnover in consumer tissues is influenced by two primary factors: growth, which involves the addition of new tissues as length and body mass increase, and metabolism, which replaces existing tissues with newly assimilated nutrients from the diet[36–40]. In large individuals, where growth is limited, isotopic turnover is primarily driven by metabolic processes. These suggest that small individuals of both sexes allocate more income resources to skeletal and

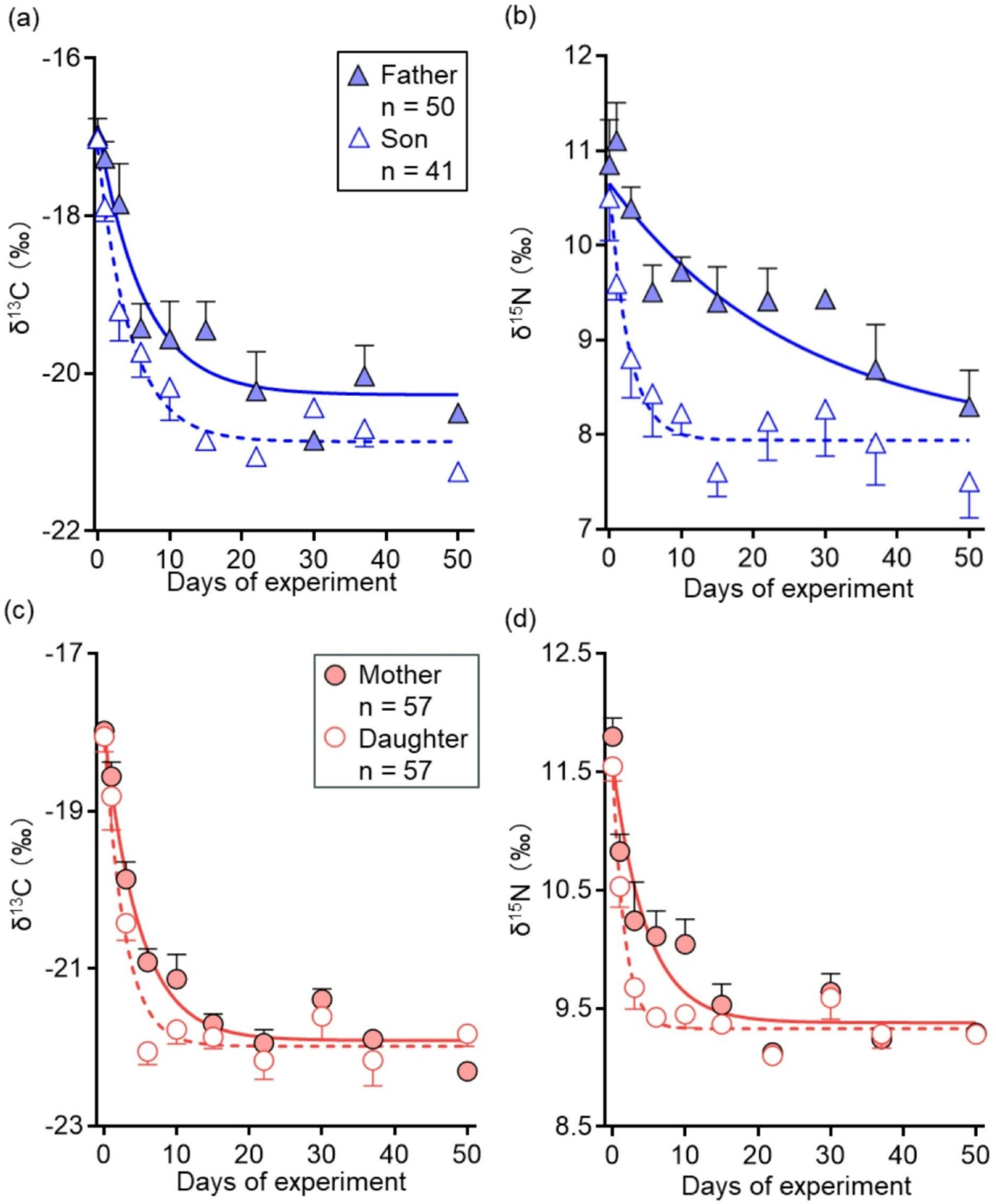

Fig. 5. Changes in $\delta^{13}C$ and $\delta^{15}N$ values (‰) of gonads in male (**a**,**b**) and female (**c**,**d**) parents (father and mother) and offspring (son and daughter) of Japanese anchovies during the experiment (days after prey change). Data are presented as mean ± standard error (SE). Exponential models fitted to the observed data were shown.

somatic growth than larger individuals during the spawning season. This is consistent with the theorical frameworks and empirical studies across fish and other taxa showing that prioritizing growth during early life stages increases the likelihood of achieving a larger body size and greater reproductive output later in life[1–7].

In males, significant differences in the TW–SW relationship between the fathers and sons were evident even within the same range of SW, suggesting that large males invest relatively more resources in sperm production than small males. In females, there was no significant difference in spawning frequency (interval of repeated spawning) between the daughters and mothers. This is probably because the processes of vitellogenesis and final

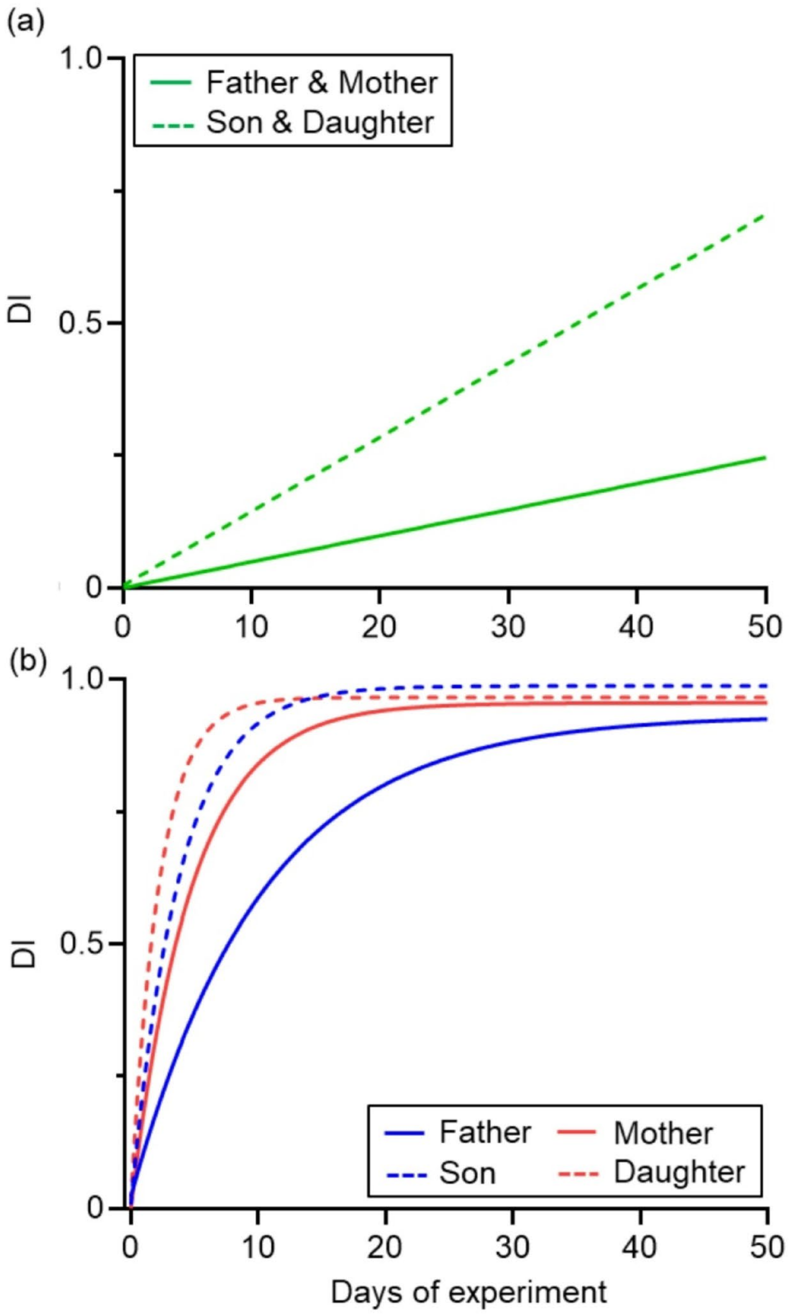

Fig. 6. Degree of dependence on income resources (DI) of muscle tissues (**a**) and gonads (**b**) of parents and offspring of Japanese anchovies during the experiment (days after prey change). DI values were derived from the modeled isotopic changes in δ^{13}C and δ^{15}N shown in Figs. 2 and 5, using linear or exponential fits. As DI values are estimated from model fits, individual data points representing variance are not available.

oocyte maturation under *ad libitum* prey availability are physiological responses to the prevailing temperature and are not influenced by body size[34]. However, the reproductive effort per spawning, calculated from the relative BF and energy per egg, differed significantly between the mothers and daughters, suggesting that large females invest relatively more resources in egg production than small females. The size (energy) per individual egg, reflecting the nutritional content and energy density, is closely related to offspring traits such as body size, growth rate, and survival under starvation in the early life stages of anchovies[41,42]. This highlights a key reproductive advantage for large females, which can produce high-quality eggs in greater numbers compared to small females, even under identical environmental conditions. In contrast, there was no direct evidence of spawning frequency in the males examined in this study. All males oozed milt under gentle abdominal pressure several hours before spawning, suggesting that they were able to participate in daily spawning events. Although differences in sperm quality, such as sperm size, swimming speed and longevity motility[12], between small and large males remain unknown, large males may have more opportunities to participate in spawning events than small males due to their enhanced sperm production capacity and potentially superior sperm storage ability, as discussed below[42].

The SIRs of the ovaries of the mothers and daughters decreased sharply during the early phase of the experiment and then plateaued, with daughters exhibiting faster turnover rates than mothers. This pattern suggests that daughters incorporated the new diet isotopes into their ovaries more rapidly, likely reflecting their higher growth rates compared to mothers. The estimated $T_{50\%}$ of $\delta^{13}C$ and $\delta^{15}N$ in daughters' ovaries were approximately 1.7 and 2.8 times faster, respectively, than in mothers. This two-phase isotopic turnover has been observed in other species and is explained by the combined effects of growth and metabolic processes[36–39].

The observed differences in isotopic turnover rates may also reflect variation in nutrient routing during vitellogenesis, as the similar spawning frequency between mothers and daughters suggests that the uptake of oocyte nutrients (e.g., vitellogenin and lipids) is consistent with the physiological processes of vitellogenesis described in teleost fish[42,44]. Therefore, the differences in isotopic turnover rates and $T_{50\%}$ of ovaries imply that the utilization of the oocyte nutrition source may differ between small and large females. Daughters rely primarily on recently assimilated nutrients, whereas mothers may integrate both their current (income resources) and stored (facultative capital resources) nutrients into developing oocytes. These findings are consistent with facultative capital breeding strategies where reproductive demands and physiological conditions influence the balance between stored and recently acquired nutrients[22–25]. The convergence of isotopic values (DI) in the ovaries of both mothers and daughters by approximately 10–20 days after the diet switch highlights the efficiency of nutrient routing into the gonads. This rapid convergence suggests that the facultative capital resources utilized by mothers were derived from recently assimilated dietary intake rather than long-term reserves accumulated prior to the spawning season, unlike capital breeders such as Atlantic herring[45,46]. This adaptability allows Japanese anchovies to maintain frequent spawning intervals efficiently.

The SIRs of the testes in fathers and sons showed patterns consistent with income breeding. Sons exhibited shorter $T_{50\%}$ of $\delta^{13}C$ and $\delta^{15}N$ than fathers, reflecting their greater reliance on current (income) resources for sperm production. Fathers, in contrast, appeared to utilize both current and stored (facultative capital) resources, a pattern also observed in large females. However, in the exponential models, there were inconsistencies in the turnover rate (k values) and plateau values between $\delta^{13}C$ and $\delta^{15}N$ of the testes in son and father. In testicular $\delta^{13}C$, fathers have significantly higher plateau values but similar turnover rates, while in $\delta^{15}N$ they have significantly higher turnover rates with similar plateau values compared to sons. This suggests possible differences in the mechanisms of carbon and nitrogen allocation. Analyses of $\delta^{13}C$ of lizard eggs indicated that the source of energy used for channeling proteins and lipids into eggs differs, although eggs are fueled by both income and capital resources[22]. Future studies could investigate the specific pathways of nutrient allocation in anchovy testes to better understand these mechanisms.

Unlike females, where oocyte nutrients are rapidly renewed during vitellogenesis and ovulation occurs every few days[47], male anchovies appear to employ a different strategy. The longer $T_{50\%}$ and slower $\delta^{15}N$ turnover rates in fathers suggest the possibility of sperm storage within the testes, allowing them to participate in daily spawning events without needing to produce large amounts of sperm immediately before each spawning. Although this study did not provide direct evidence of prolonged sperm storage, previous findings indicate that the mature testes of male anchovies accumulate substantial amounts of sperm while spermatogenesis occurs concurrently[48]. This implies that males may rely on a balance between active sperm production and storage to sustain reproductive participation across the spawning season.

Given the schooling behavior of anchovies, where multiple males compete for fertilization, conserving sperm for successive reproductive opportunities could be advantageous. This is consistent with the theory of sperm competition, wherein males modulate their ejaculate expenditure in response to the intensity of competition, thereby optimizing reproductive success over time[12,49]. Such a strategy may explain the observed differences in isotopic turnover dynamics between males and females, with males exhibiting slower rates due to the combined effects of sperm retention and metabolic processes, the latter associated with sperm maintenance, such as ATP production for motility and viability[50,51].

There are several mechanisms by which female size and age influence their contribution to recruitment[10,11]. Our findings showed that large spawning individuals of both sexes allocate more resources to reproductive output than small individuals, consistent with patterns observed in many organisms[6,7]. Small spawning individuals, on the other hand, appeared to prioritize skeletal and somatic growth, a strategy that likely facilitates rapid attainment of larger body size. This growth-focused strategy during early reproductive life could improve the long-term quantity and/or quality of gametes, enhancing reproductive success later in life[1,2].

The dynamics of SIRs (DI) in the muscles and gonads suggest that spawning individuals of both sexes exhibit temporal flexibility in their use of income resources for gamete production. This flexibility allows them to allocate less to body growth during the spawning season while maintaining gamete production. In *E. japonicus*, insufficient intake of income resources may slow oocyte growth relative to the prevailing temperature, prolonging vitellogenesis[34]. Females in such nutritionally unfavorable environments may maintain reproductive effort per spawning event at the cost of reduced spawning frequency. Conversely, large females with access to facultative capital resources can sustain continuous spawning, buffering against short-term prey and thermal fluctuations. This bet-hedging strategy provides a reproductive advantage in unpredictable environments by ensuring consistent reproductive output. Similarly, large males may benefit from facultative capital resources by maintaining sperm production and storage, allowing them to participate in daily spawning events even under fluctuating environmental conditions. Small males, heavily reliant on current resources, might face constraints in sperm production during periods of limited food availability. While small individuals may adapt more quickly to favorable changes in environmental conditions, this adaptability could be constrained by their simultaneous need to invest in growth and reproduction. Thus, size-related reproductive strategies in both sexes reflect adaptive responses to environmental variability, optimizing lifetime reproductive success across different life stages and conditions.

In conclusion, this study demonstrates that spawning Japanese anchovies of both sexes exhibit reproductive tactics influenced by body size, not only in the quantity and quality of gamete production but also in how repeated spawning is maintained. Large individuals showed adaptive bet-hedging strategies, allowing them to sustain reproductive output in fluctuating environments, thereby promoting stock productivity and stability. These findings contribute to our understanding of individual-level life history responses and the population dynamics of aquatic organisms with external spawning.

Meanwhile, we acknowledge some limitations that point to potential directions for future research. The 50-day laboratory experiment provided a controlled perspective but did not encompass seasonal or interannual variations in reproductive strategies. Additionally, while the absence of lipid extraction might have slightly influenced δ^{13}C values[52], particularly between size classes with differing lipid content[53], it does not detract from the primary findings of this study due to our focus on comparing SIRs turnover across different sizes and sexes.

Our findings highlight the importance of size- and sex-specific energy allocation strategies in Japanese anchovies and provide a framework for studying other small pelagic species. For example, European sardine (*Sardina pilchardus*) and European sprat (*Sprattus sprattus*) are thought to follow mixed income-capital breeding strategies[54,55], but the role of size and sex in these tactics remains unclear. Applying stable isotope analyses to these species could uncover broader resource allocation patterns. Future studies could also incorporate models that distinguish isotopic turnover due to growth and metabolism[39,40,43] to enhance our understanding of the mechanisms driving size- and sex-specific differences. These efforts would expand the applicability of our findings and further elucidate the adaptive strategies of small pelagic fish, contributing to a deeper understanding of their life history traits.

Methods

Ethical statement

All experimental procedures followed the Guidelines for Animal Welfare of the Fisheries Research and Education Agency, Japan (50322001) and were approved by the Committee of Animal Welfare of the National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research and Education Agency. In addition, this study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Fish rearing

This section describes the acclimation conditions for the parents and offspring prior to the diet-change experiment. Anchovies were caught using purse sein fishing with light assistance in Omura Bay, Nagasaki Prefecture, western Japan, and maintained for a few weeks in the sea cages of a fishing company (Takeshita Suisann, Nagasaki, Japan). A total of about 700 specimens were transferred to the Hakatajima Field Station of the National Research Institute of Fisheries and Environment of Inland Sea in February 2014. To monitor spawning of large individuals (parents), a total of 100 individuals were kept in 2 m^3 circular tank. This setup was replicated twice, resulting in two tanks each containing 100 individuals. The tanks were maintained at ambient water temperature under 14/10- light/dark photoperiod in an indoor rearing facility. The remaining specimens were kept in 20 m^3 concrete tank under the same environmental conditions in an indoor rearing facility. The water temperature in the tank was recorded every hour using a data logger (TidbiT® v2 Temp Logger; Onset, Bourne, MA, USA). The fish were provided daily *ad libitum* access [3% of their body weight (BW)] to commercial dry pellets with high SIRs (-19.1‰ for δ^{13}C and 11.3‰ for δ^{15}N, 50% protein, 10% oil, 16% ash, and 3% fiber; Otohime C2, Marubeni Nisshin Feed, Tianjin, China) with the start of the experiment (in October 2014). The δ^{13}C and δ^{15}N compositions of the commercial feeds were analyzed following the method described below. Dead fish were counted daily to adjust the food intake ratio in each tank.

To produce offspring, eggs were collected from the parent on May 27, 2014. Approximately 10,000 eggs were kept in 2 m^3 rearing tank with two replicates. The offspring were reared under a 14/10-h light/dark photoperiod. Approximately 20–25 ml^{-1} of S-type rotifer *Brachionus plicatilis* complex cultured with highly unsaturated fatty acid–enriched *Chlorella vulgaris* (Super Fresh Chlorella V12; Chlorella Industry, Tokyo, Japan) were provided to the offspring from age 2 to 50 days. Rotifer density was monitored daily and maintained by adding new rotifers if necessary. Approximately 0.2–1.5 ml^{-1} of *Artemia* sp. nauplii enriched with highly unsaturated fatty acids (Super Capsule Powder, Chlorella Industry, Tokyo, Japan) were provided to the offspring from age 30 to 50 days, and commercial dry pellets with the same SIRs levels as those fed to the parents were provided daily at 0.5–3.0% BW after age 50 days. From 80 to 130 days after hatching, 16–20 females were randomly selected from the tanks at intervals of 6–14 days to monitor the maturational condition of the offspring because the onset of ovarian maturation occurs later than testicular maturation at a given temperature[48]. After the females were killed with 2-phenoxyethanol (300 ppm), their ovaries were removed. To determine the developmental stage of the oocytes, the location of the nuclei was confirmed by placing small pieces of the ovarian subsamples in approximately 500 µL of clearing solution (ethanol: formalin: ascetic acid = 6:3:1)[47] to make the cytoplasm transparent. Mature females were determined as those having vitellogenic oocytes. The maturation rate of the females increased from age 80 to 130 days, and 88% of the females were mature at age 130 days (Supplementary Fig. S1).

Experimental setup

This section outlines the setup and sampling methods used during the diet-change experiment. The diet-switch experiment lasted for 50 days (October 7 to November 26, 2014). On October 7, 2014, 100 fish specimens randomly selected from 20 m^3 concrete tank (parents) and 2 m^3 rearing tanks (offspring) were moved into 2 m^3 circular tanks at an ambient temperature under 14/10-h light/dark photoperiod in an indoor rearing facility; there were 2 replicates per treatment (parents and offspring). Offspring were at 131 days post-hatch at the beginning of the experiment. Commercial dry pellets with low SIR levels (−23.3‰ for δ^{13}C and 9.1‰ for δ^{15}N, 53% protein, 8% oil, 15% ash, and 3% fiber; Torafugu EP, Marubeni Nisshin Feed, Tokyo, Japan) were provided *ad libitum* (3% of body weight, BW) daily. Sampling intervals varied (1–18 days) to capture rapid isotopic turnover at the beginning and slower changes as tissues approached isotopic equilibrium[36–38]. A total of 19–47 specimens per treatment were randomly collected from the tanks at each interval (Supplementary Table S7). In the initial phase, the number of sampled individuals was minimized to focus on gonadal turnover. Conversely, in the latter phase, a larger number of individuals was collected to investigate spawning traits. Sampling was conducted around 3:00 pm to determine the number of females that spawned at night (ovaries with migratory nucleus) based on the daily ovarian maturation cycle of anchovies[47]. The male specimens whose milt oozed when gentle abdominal pressure was applied were considered mature. After the specimens were killed with 2-phenoxyetahol, their standard length (SL, mm), BW, and testicular weight (TW, g) or ovarian weight (OW, g) were measured to the nearest 1 mm, 0.1 g, and 0.01 g, respectively. Somatic weight (SW, g) was computed by subtracting TW or OW from BW. The ovary samples were fixed in 10% neutral formalin to determine maturational conditions using histological observations[47,48] and to estimate batch fecundity (BF).

Reproductive traits

The relationship between the TW and SW of the mature males was examined[15]. To compare the spawning intervals between the parent (mother) and offspring (daughter), spawning frequency was estimated as the ratio of females with ovaries having hydrated or migratory nuclei oocytes to the total number of females collected on the day of sampling. To compare the number of eggs spawned by the parent (mother) and offspring (daughter), a gravimetric method was employed to estimate BF from the specimens with ovaries having hydrated or migratory nuclei oocytes[34,47]. The BF of each female was calculated as the number of hydrated and migratory nuclei oocytes per unit weight multiplied by the OW.

The dried egg weight (DEW) represents the average dry weight of a single egg (mg), measured after drying pooled approximately 250 eggs samples at 60 °C for 3–4 days. The DEW value is calculated by dividing the total dry weight of a batch by the total number of eggs in the batch. This metric allows to evaluate energy investment per egg. To compare the reproductive effort per spawning between the parent (mother) and offspring (daughter), the relationships between the DEW and the carbon (C, μg) and nitrogen (N, μg) content of an egg[34] were calculated using Eqs. (1) and (2):

$$\mathrm{C} = \exp\{6.47 + ln\,(\mathrm{DEW}) \cdot 10.043\}\ (\mathrm{R}^2 = 0.99,\ \mathrm{n} = 63) \tag{1}$$

$$\mathrm{N} = \exp\{4.69 + \mathrm{l}n\,(\mathrm{DEW}) \cdot 0.976\}\ (\mathrm{R}^2 = 0.98,\ \mathrm{n} = 63) \tag{2}$$

As eggs of marine fish species, including anchovies, primarily consist of lipids and proteins[41,42,44], the carbon and nitrogen content of an egg are considered to be as those of lipids and proteins. Energy content was calculated by applying energy equivalents of 39.5 J/mg for lipids and 23.6 J/mg for proteins[53]. The reproductive effort (J) of the females was determined as the estimated nutritional energy of eggs per spawning:

$$\text{Reproductive effort} = \mathrm{TC} \cdot 39.5 + \mathrm{TN}23.6 \tag{3}$$

where TC and TN are the total carbon and nitrogen content of the eggs to be spawned, respectively; TC and TN were calculated by multiplying the carbon or nitrogen content by the relative BF (BF per SW).

Stable isotope dynamics

Muscle and gonadal samples were frozen at −20 °C prior to analysis. After thawing the muscle and gonads at room temperature, they were rinsed with distilled water. All the samples were freeze-dried in microtubes. The dried samples were ground to fine powder, and the powdered samples (approximately 0.5–1.0 mg) were placed in tin capsules. Lipids were not removed to assess the full complement of nutrients, as this study focused on the dynamics of stable isotope turnover rather than absolute δ^{13}C values. C and N SIRs were analyzed using an SIR mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, Inc., USA) in continuous-flow mode, equipped with an elemental analyzer Flash 2000, Thermo Fisher Scientific Inc, USA). The SIRs were expressed as per mil (‰) deviations from the standard as defined by Eq. (4):

$$\delta^{13}\mathrm{C}, \delta^{15}\mathrm{N} = (\mathrm{R}_{\mathrm{sample}}/\mathrm{R}_{\mathrm{standard}} - -1) \times 1000 \tag{4}$$

where, R is the ^{13}C/^{12}C or ^{15} N/^{14}N ratio. PeeDee Belemnite and atmospheric N_2 were used as the standards for C and N, respectively. $_{\mathrm{L}}$-Alanine was used as a secondary standard in the laboratory, and the standard deviations of the measured δ^{13}C (±0.1‰) and δ^{15}N (±0.2‰) values for the secondary standard were consistently within acceptable ranges.

Statistical analysis

Statistical analyses were performed using Prism GraphPad version 8.4.3[56] and R version 4.1.2[57]. To compare the biological traits between parents and offspring, a linear mixed-effects model (LMEM) with a restricted maximum likelihood was fitted to the data on the day of the experiment. Initially, the model with the minimum conditional Akaike Information Criterion (cAIC)[58] was selected from multiple full models, including those with and without natural logarithmic transformation. Subsequently, the significance of each variable was examined, and non-significant variables were removed sequentially using the step function in the R package car. After each removal, the cAIC was re-evaluated to ensure the model remained optimal. This process was repeated until only significant variables remained, and the final model had the lowest cAIC among all considered models.

To compare changes in the SIRs of the muscles and gonads between the parents and offspring, an LMEM or exponential model (one phase decay) was fitted to the data on the day of the experiment based on the minimum cAIC. A one phase decay model implemented in Prism GraphPad[56] and the equation used is:

$$\mathrm{Y_t} = \mathrm{Y_0}\cdot\exp(-\mathrm{k}\cdot\mathrm{t}) + \mathrm{Plateau} \qquad (5)$$

where Y_t is the isotopic ratio ($\delta^{13}C$ or $\delta^{15}N$) at experimental day (time) t, Y_0 represents the difference between the initial isotopic ratio (δ_0) and the equilibrium value (Plateau), and k is the rate constant for isotopic turnover. The half time required for its initial difference from equilibrium ($T_{50\%}$) is calculated as:

$$\mathrm{T_{50\%}} = ln\,(2)\,/\mathrm{k} \qquad (6)$$

This one phase decay model is conceptually consistent with the exponential models described by Tieszen et al. (1983)[36] and Hesslein et al. (1993)[37], which similarly describe isotopic turnover as a function of time and include contributions from growth and metabolic turnover. For the one phase decay model, the D'Agostino–Pearson normality test was performed to determine whether the residuals of the data followed a Gaussian distribution. All data used in this model passed the normality test.

The effect of income resources on growth and reproductive output in terms of the capital–income breeding continuum was estimated[21]; the DI was estimated using Eq. (7):

$$\mathrm{DI} = (\delta\mathrm{X_{end}} - \delta\mathrm{X_0})\;/\;(\delta_\mathrm{L} - \delta_\mathrm{H}) \qquad (7)$$

where δX_{day} is a predicted value of muscle and gonads calculated from a linear or exponential regression model, and δ_H and δ_L are the values of high and low SIRs that dry pellets provide. It is assumed that DI would be close to 1.0 if gamete production during the experiment depended mostly on income resources, reflecting the SIR of diet switch, whereas DI would be close to 0 if it depended largely on capital resources (no significant changes in the SIR of eggs independent of diet switch). To model the dynamics of DI over time, a one-phase association model was applied, mathematically consistent with the one-phase decay model but describing an increasing relationship toward equilibrium. The equation used is:

$$\mathrm{DI_t} = \mathrm{Plateau}\cdot(1 - \mathrm{expt}(-\mathrm{k}\cdot\mathrm{t})) \qquad (8)$$

where DI_t represents the DI at experimental day (time) t, Plateau is the asymptotic DI value, and k is the turnover rate. This approach is consistent with established frameworks[28,39] and captures the temporal progression of isotopic incorporation during the diet-switch experiment.

Data availability

The datasets generated and analyzed during the current study are available in the figshare repository, accessible via the following link: 10.6084/m9.figshare.27998438.

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Author contributions

M. Yo., S. K. and M. Ya. designed the study. M. Yo. and T. T. performed the experiments. M. Yo. and S. K. analyzed the experimental data. M. Yo. wrote and edited the manuscript. S. K., M. Ya., N. K. and H. T. gave beneficial advice. All authors have reviewed the manuscript and approved the submitted version.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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