

Ontogeny of predatory capacity and prey choice during early life of the holobenthic octopus *Amphioctopus fangsiao* (d'Orbigny, 1841): switching prey-choice strategy

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Abstract

Benthic octopuses are well known generalist predators but the predatory behavior of juveniles is poorly understood. This study investigated prey choice of juvenile *Amphioctopus fangsiao*, a holobenthic species. Juveniles aged 0 to 50 d after hatching (0.1 – 5.2 g wet weight) were offered seven individuals of each of three types of prey organism: bivalves, gastropods, or mysids. The juveniles consumed all three species from the day of hatching (0 d) but the energy source of the early juveniles relied largely on gastropods. Juveniles could catch gastropods easily by detaching them from the bottom of the tank but required a longer time to consume them. Their ability to pull open bivalve shells and to successfully catch mysids developed as they grew, and the grown juveniles chose mysids and bivalves as their main prey organisms. Early juveniles of < 2.5 g tended to choose prey individuals from which they could obtain more energy. However, grown juveniles of > 2.5 g changed the criteria of prey choice to attain a shorter time of prey consumption and a faster rate of energy intake. These suggest that *A. fangsiao* has a switching prey-choice strategy: early juveniles give priority to growth, but grown juveniles give priority to reduce time suffering predation risk while obtaining energy quickly as much as possible. Because early juveniles can develop the predatory capacity as they grow faster, they seem to take a strategy to invest growth to go through quickly the period of high risks of predation and starvation.

Introduction

For carnivorous animals, successful predation on their prey determines their survival and growth. Since prey animals have evolved various antipredator responses (Begon et al. 2011), the young of carnivorous animals are at risk of an early death by starvation if their predatory skills are insufficient. This is likely to be true of octopuses, which are well-known predators in marine benthic communities. Adult octopuses living in shallow waters can consume various animals such as crabs, shrimps, lobsters, gastropods, bivalves, polychaetes, and teleost fishes (Ambrose 1984; Iribarne et al. 1991; Vincent et al. 1998; Smith 2003; Anderson et al. 2008; Mather et al. 2012; Scheel and Anderson 2012; Hofmeister 2018; Bo et al. 2020).

Among benthic octopus species, there are two types of life cycle strategy: one is the merobenthic type having planktonic paralarval phase before taking up the benthic life, and the other is the holobenthic type that commences benthic life immediately after hatching (Villanueva and Norman 2008; Vidal and Shea 2023). Although the hatchlings of both reproductive types are miniatures of the adult form showing typical carnivorous behavior, the hatchlings of holobenthic octopuses possess relatively well-developed morphological traits such as longer arms with dozens of suckers and more chromatophores compared with those of merobenthic species. Nevertheless, the juveniles of holobenthic octopuses must explore, seize, and ingest prey organisms immediately after hatching (and without any parental support) in a marine benthic habitat in which they compete with various cohabiting carnivorous animals. Since octopuses have no protective shell, it is assumed that juveniles are at high risk of predation from predators, including conspecifics, as well as at risk from starvation if they are unable to obtain sufficient prey on which to feed. In this context, young octopuses must develop their predatory capacity as soon as

possible to maximize their energy intake while minimizing their time at risk of predation (Mather et al. 2014). The ontogeny of predatory capacity can be considered as one aspect of antipredation strategy. To reveal how they overcome these conflicting requirements is very important to understand their adaptive strategy during early life. Few studies have investigated the predatory capacity of octopus juveniles, and its ontogenetic development is poorly understood (Villanueva et al. 2017).

Optimal foraging theory is a well-known assumption that provides a model to assess the systematic trends of animal feeding behavior: the animals are predicted to adopt the feeding behavior that maximizes the energy intake per unit time, represented as E/H , where E is energy content obtained from an individual prey animal and H is the handling time required to obtain energy from the prey (Pyke et al. 1977; Stephens and Krebs 1986; Stefens et al. 2007). Regarding the feeding behavior of octopuses, it is well recognized that, among their possible prey animals, they have a clear preference for crabs (Onthank and Cowles 2011; Hanlon and Messenger 2018; Markaida 2023).

Octopuses will also consume bivalves and can use alternative methods to gain access to the animal inside, separating the two shell valves either by just pulling them apart using the arms and suckers, or by a combination of drilling and pulling (Nixon 1979, 1980; Fiorito and Gherardi 1999; Steer and Semmens 2003; Fee et al. 2023). Drilling requires a longer H to penetrate the hard shell using a specialized tooth-bearing salivary papilla and enzymes secreted by the posterior salivary glands (paralysing the animal, which relaxes and allows the valves to be parted), whereas pulling requires more energy input (E) to open the valves by force alone in comparison to combining pulling with drilling (Steer and Semmens 2003; Ebisawa et al. 2011). In laboratory experiments with *Octopus vulgaris* and *Amphioctopus fangsiao*, the octopuses tended to use these methods differently depending on prey size to maximize E/H (McQuaid 1994; Ebisawa et al. 2011). In experiments with *Octopus minor*, bivalve shells were not opened by drilling, only by pulling (whichever species was supplied), and this species of octopus preferred prey species with a higher E/H (Song et al. 2019).

These findings suggest that octopuses are “rate-maximisers” (giving priority to higher E/H) in terms both of prey choice and the decision of which method(s) to use to consume prey. In field observations with *O. vulgaris* and *O. insularis*, the octopuses spent much time in their den and a relatively short time out foraging, while showing cryptic body patterns during foraging and irregular movement across the habitat (Mather and O’Dor 1991; Leite et al. 2009). This suggests that these species are “time-minimizers” when foraging, preferring to give priority to minimizing H . That is, octopuses seem to possess different strategies for prey consumption versus actively foraging and feeding. Nevertheless, it is noteworthy that both strategies share a common decision criterion to shorten the time (H) spent in foraging and consumption rather than focussing on maximizing energy intake (E). Taking their vulnerable soft body into account, it is likely that octopuses give priority to survival (avoiding predators) by reducing time suffering predation risks rather than maximizing their growth (Mather et al. 2014). Predation risk is likely to be higher for juveniles than adults, in a size-dependent manner (Anderson 1988; Urban 2007). This implies that predation avoidance is more important for juveniles, with predation risk decreasing as the juveniles grow. Thus, for juveniles there is an unavoidable trade-off between the risks of foraging and

feeding versus maximizing growth (Lankford et al. 2001; Biro et al. 2006; Urban 2007). Prey choice is expected to be closely related to the strategy for dealing with this trade-off, so it is important to learn more about the criteria of prey choice and any ontogenetic changes during the early life of octopuses.

To achieve a fundamental understanding of the ontogenetic development of predatory behavior and prey choice during the early life of holobenthic octopuses, it is necessary to test their predatory behavior on a selection of potential prey animals of various sizes and species involving crustaceans, bivalves, and gastropods, which are known to be the major prey organisms sought by octopuses (Ambrose 1984; Hanlon and Messenger 2018). In testing the choices of octopuses, prey type (species and size) and the rank order in which prey are captured and consumed are decision variables based on predatory skills and prey choice to minimize risk (decrease H), maximize growth (increase E), or strike a balance (optimize E/H). To obtain consistent choices by the octopus while eliminating the variability introduced by active foraging, the availability and sensory information concerning prey items should be maintained constant (Stefens and Krebs 1986).

The present study used *A. fangsiao* as a model holobenthic octopus, because this species is well known to commence benthic life immediately after hatching and the juveniles consume crustaceans and molluscs (Kitajima and Hayashida 1985; Segawa and Nomoto 2002; Ebisawa et al. 2011). It has a relatively small adult body size of approximately 120 g wet weight (WW) when mature (spring) and a short life span of about a year (Wang et al. 2015; Yamamoto and Abe 2022). Newly hatched *A. fangsiao* juveniles were reared for 50 d, and seven individuals of each of juvenile clams (bivalve molluscs), snails (gastropod molluscs), and mysids (crustaceans) were supplied to isolated juvenile octopuses as prey organisms under the same experimental conditions for 24 h every five days, and their feeding behavior was recorded. The findings describe the development of predatory skills and choice of prey by this species, illustrating the early days of their coordination of growth and survival against the risks of starvation and falling prey to their predators.

Materials and Methods

Animals

Six wild female *Amphioctopus fangsiao* (d'Orbigny 1841) were caught using a trawl net on 2 February 2021 in the central area of the Seto Inland Sea (34°25'N, 133°47'E), off Kurashiki City, Okayama Prefecture. They were packed individually in a plastic bag with seawater and oxygen gas, then transported by a bullet train (Shinkansen) to Tokyo University of Marine Science and Technology. Body wet weight (WW) of the females was 125 ± 32 g (range 75 – 162 g). They were individually maintained in a 40 L rectangular glass aquarium (450 × 300 × 300 mm) containing artificial seawater (Sealife 714; Nihonkaisui Co., Ltd., Tokyo, Japan) equipped with a water recirculation system (ProFit Filter Big; Kotobuki Kogei Co., Ltd., Nara, Japan). Frozen shrimp (*Plesionika semilaevis*) were supplied to the females once a day until the start of spawning. Water temperature, pH, dissolved oxygen concentration, light intensity, and salinity were measured once a day at 9 a.m. The respective mean values ($\pm$ SD) were

as follows: $11.4 \pm 1.8^{\circ}\text{C}$, $\text{pH } 8.08 \pm 2.19$, $11.0 \pm 0.5 \text{ mg L}^{-1}$, $99.0 \pm 43.7 \text{ lx}$, and $32.3 \pm 1.3 \text{ g L}^{-1}$. Females started spawning from 25 March 2021, and were left (without further feeding) to incubate their eggs until hatching.

Seven hatchlings (mean WW $0.12 \pm 0.03 \text{ g}$, range $0.08 - 0.15 \text{ g}$; hatched during the period 23 to 30 June 2021) were reared individually in cylindrical plastic containers (diameter 70 mm, length 105 mm) in which numerous small holes (diameter 1 mm) allow free circulation of seawater. These containers were placed together in a water bath filled with artificial seawater ($500 \times 807 \times 207 \text{ mm}$, water volume 60 L). To prevent escapes, the upper aperture of the container was occluded with a polyurethane sponge. Small frozen shrimps (*Acetes japonicus*) were supplied twice a day as food for these juveniles. The rearing water was changed once a week, and the water temperature, pH, dissolved oxygen concentration, light intensity, and salinity were measured once a day at 9 a.m. during rearing periods, with the following respective values (mean $\pm$ SD): $22.4 \pm 1.5^{\circ}\text{C}$, $\text{pH } 8.26 \pm 0.11$, $9.3 \pm 0.3 \text{ mg L}^{-1}$, $127.3 \pm 120.7 \text{ lx}$, and $32.9 \pm 1.37 \text{ g L}^{-1}$. Juvenile rearing was terminated at 50 d after hatching, by which time the WW of the juveniles had reached $4.69 \pm 0.50 \text{ g}$ (range $3.86 - 5.22 \text{ g}$, Fig. S1).

As experimental prey organisms for *A. fangsiao* juveniles, juvenile clams (the bivalve *Ruditapes philippinarum*), small marine snails (the gastropod *Littorina brevicula*), and mysids (the mysid shrimp species *Neomysis awatschensis*) were selected, because they are sympatric with *A. fangsiao*. The juvenile bivalves and gastropods were captured, respectively, at a tidal flat ($35^{\circ}20'\text{N}$, $139^{\circ}38'\text{E}$) and a rocky shore ($35^{\circ}18'\text{N}$, $139^{\circ}29'\text{E}$) in Tokyo Bay, Kanagawa Prefecture, Japan (permission number 3–40, 2021, Kanagawa Prefecture). The mysids caught from the coastal waters of Kyusyu Island of Japan were purchased from an aquarium trader. These prey organisms were maintained in aquaria with a supply of appropriate feed: microalgae *Chlorella vulgaris* (fortified with n-3 unsaturated fatty acids) for juvenile bivalves; pieces of kelp for the gastropods; and *Artemia franciscana* nauplii and artificial compound diet (Vital Prawn S0, Higashimaru Co., Ltd., Kagoshima, Japan) for the mysids.

Feeding experiment

To investigate ontogenetic changes in predatory capacity and prey choice of *A. fangsiao* juveniles, seven octopuses were used for the feeding experiment. The experiment was carried out in an experimental arena on the day of hatching (aged 0 d) using four juvenile octopuses (octopuses A – D, Fig S1) and then repeated every five days until the juveniles were 50 d old (11 times per juvenile, for a total of 44 times). Three additional octopuses (E – G) were used as substitutes on three occasions, following failure to obtain results (such as the loss of power supply to the video camera or escape of the octopus from the experimental arena).

The experimental arena consisted of a cylindrical acrylic container placed concentrically inside a larger container (Fig. 1). Both the outer (inner diameter 292 mm, height 100 mm) and inner (inner diameter 144 mm, height 50 mm) containers were filled with artificial seawater (water depth 55 mm). To prevent escapes, nylon mesh (height 70 mm) was attached to the inside wall of the inner container, and an air

stone was placed in the outer container to provide oxygen while recirculating the water between the inner and outer containers.

Seven individuals of each of the three prey organisms (total 21 animals) were placed in the experimental arena and then the octopus was added inside a 100 mL glass beaker placed in the center of the arena for acclimation. During the acclimation period (10 min), prey organisms were able to move freely around the arena and the octopus could see them but could not contact them. Then, the octopus was released from the beaker and its behavior was recorded for 24 h. A video camera (HDR-SR12; Sony Corporation, Tokyo, Japan) was placed facing upward in a space beneath the arena and the octopus's behavior was recorded through the translucent acrylic base of the arena (Fig. 1). White LED lights illuminated the roof (white PVC board) of the aquarium and were programmed to produce a photoperiod of 13L:11D. During the dark period (from 7 pm to 5 am), the white LEDs were replaced with an infrared LED. The video camera was set to infrared mode throughout the entire experimental period (for 24 h). The camera was connected to a video capture board (GV-HDREC; I-O Data Device Inc., Kanazawa, Japan) and HDD recorder (ELP-CED020U; Elecom Co., Ltd., Osaka, Japan). The octopus was removed immediately after the experimental period, and its WW was measured before returning it to the rearing container. Water temperature, pH, dissolved oxygen concentration, light intensity, photon flux density, and salinity of the experimental aquarium were measured immediately after finishing the 24 h video recording, producing the following respective mean values: $22.4 \pm 1.0^{\circ}\text{C}$, $\text{pH } 8.25 \pm 0.12$, $8.95 \pm 0.35 \text{ mg L}^{-1}$, $102 \pm 11.1 \text{ lx}$, $2.65 \pm 0.51 \mu\text{mol m}^{-2} \text{ sec}^{-1}$, and $32.3 \pm 1.36 \text{ g L}^{-1}$.

As prey organisms, seven individuals each of bivalves and gastropods of various sizes were concomitantly supplied for the experiment. The mysids presented as prey were randomly sampled from the mysid-rearing tank. The size of prey organisms was measured using a stereomicroscope equipped with image analysis software (NIS-Elements D5.10.00; NIKON Corporation, Tokyo, Japan). The shell length (SL) of the bivalves supplied to the octopuses was $6.98 \pm 2.34 \text{ mm}$ (range 2.21–12.24 mm), and the shell height (SH) of the gastropods was $6.43 \pm 0.95 \text{ mm}$ (3.81–9.59 mm). The experimental period ended when the octopus was removed. The number of surviving prey organisms was counted and the size of consumed bivalves and gastropods was measured. The total length (TL) of surviving mysids was also measured (mean TL $8.20 \pm 0.89 \text{ mm}$; range 6.06–10.64 mm).

Video-recorded behavior of the octopuses during 44 trials (total 1056 h) was observed using media player software (VLC ver. 3.0.15). The time (in seconds) that the octopus spent consuming individual prey organisms, from capture to discarding (shells or exoskeletons), was recorded as handling time (H). Where an octopus discarded a prey organism with a large amount of residual flesh, and H was obviously short, the value of H was excluded from the data to avoid overestimation of the predatory capacity of the octopus. The rank order in which each prey organism was consumed by each octopus was also recorded and the size of bivalves and gastropods was measured. To assess the predatory capacity of octopuses on mysids, the number of attacks on mysids was counted to calculate the proportion of successful attacks.

Force required to prey on bivalves and gastropods

To quantify the predatory capacity of octopuses needed to prey on bivalves and gastropods, the forces required to open bivalve shells and to detach gastropods from the substrate were investigated. Live bivalves (55 individuals with a size range of 2.21–12.88 mm SL) were used. After SL measurement, one valve was attached to a polyethylene thread and the other valve was firmly attached to an aluminium block using instant glue (Aron Alpha Extra; Toagosei Co., Ltd., Tokyo, Japan). The aluminium block was secured to a table with clamps and the thread was connected to a force gauge (AD-4932A-50N, A&D Co., Tokyo). The maximum force (unit N) required to open the bivalve was measured by pulling the force gauge. The force required to detach each gastropod was also measured using a force gauge. Live gastropods (27; size range 4.73–9.64 mm SH) were used. A polyethylene thread was attached to the top of the gastropod shell with instant glue, then the gastropod was put into the arena (the same as that used for the feeding experiment). After ensuring that the foot of the gastropod was adhering to the base of the arena, the force gauge connected to the thread was pulled up to detach the gastropod and the maximum force used was recorded.

Energy content of prey organisms

To estimate the energy content of the prey organisms, chemical analysis was conducted on their flesh. The whole body of mysids and the soft bodies of bivalves and gastropods were sampled and preserved at -60°C until analysis. The content of moisture, crude protein, total lipid, and ash were determined by, respectively, the air-oven method, combustion method, acid hydrolysis method, and direct method (ashing at 550°C). Carbohydrate content was calculated by the moisture, crude protein, total lipid, and ash content. Energy content (unit J) was estimated based on the protein, lipid, and carbohydrate contents in dry matter basis (Table S1). These were the standard analysis method for food composition in Japan (Ministry of Education, Culture, Sports, Science and Technology, Japan 2020).

The relationship between size and dry weight (DW) of gastropods and mysids was also assessed. For gastropods, the soft bodies of 20 individuals were dissected from the shell after measuring SH, and the DW of the soft body was measured by drying at 100°C for 24 h. For the mysid, the DW of the whole bodies of 5 individuals was determined for a total of 100 individuals. For the bivalves, the DW of the soft body was estimated from the shell size (SL) according to Hasegawa and Higano (2010): $DW = 3.6 \times 10^{-6} \times SL^{3.30}$.

Data analysis

All statistical analyses were performed using R statistical software (R4.3.2 Core Team 2023) with a 5% significance level. Log-transformed values of weight (DW and WW), length (SL and SH), and force were used to standardize their additivity in the models.

To determine the relationship between gastropod DW and SH, a linear model (LM) was applied using the *lm* function, while DW and SH were used as response and explanatory variables, respectively. A LM was

also applied to estimate the relationship between shell size (SL or SH) and force required to open bivalves or detach gastropods.

To estimate the relationships between the size of octopus and sizes (SL and SH) of the bivalves and gastropods consumed by the octopus, a linear mixed-effect model (LMM) was applied using the *lmer* function in the lme4 package (Bates et al., 2015). In this analysis, mean or maximum shell sizes, consumed by an octopus during the test period of 24 h, were used as response variables. When the octopus consumed one bivalve or gastropod, the size was treated as a mean (not a maximum) value. The explanatory variable was the WW of the octopus, and the identity (A – G) of the octopuses was included as a random intercept effect (Zuur et al. 2009). The statistical significance of the explanatory variable was evaluated using the *Anova* function (type II, F tests) in the 'car' package (Fox and Weisberg 2011). To assess the change in the success ratio of attacking to catching of mysids by the octopus, a generalized linear mixed effect model (GLMM) with the binomial family (logit link) was applied using the *glmer* function in the lme4 package. In this analysis, the number of successful and unsuccessful attacks were binary response variables, and the WW of the octopus was an explanatory variable, while the identity of the octopus was included as a random intercept effect.

To examine changes in time (H) spent to consume individual prey organisms, a GLMM with the Gamma family (log link) was used. In this analysis, handling time to consume individual bivalve (H_b), gastropod (H_g), and mysid (H_m), were used as separate response variables in three GLMMs, while octopus WW and prey size (SL or SH) were used as explanatory variables. For mysids, prey size was excluded from the model because individual mysids were closely similar in size. The Gamma GLMM was used to assess the octopus rate of energy intake per unit time (i.e., E/H). In this model, the E of an individual bivalve (E_b) or gastropod (E_g) were used as separate response variables in two GLMMs, while the H_b or H_g were used as an offset. The octopus WW was an explanatory variable. In these GLMMs, the identity of the octopus was included as a random intercept effect. The mysids were of similar size, so it was assumed that their energy content (E_m) was similar and the E_m/H_m ratio for mysids was estimated simply using the mean E_m of individual mysids and the relationship between octopus WW and time H_m spent to consume the mysid.

To assess the effects of octopus size on total time spent to consume prey organisms (H_t , summation of all H_b , H_g , and H_m) and total intake of energy (E_t , summation of all E_b , E_g , and E_m) during the 24 h experimental period, GLMMs with the Gamma family (log link) were applied. In these analyses, values of H_t or E_t were the response variables; and the WW and identity of the octopuses were an explanatory variable and a random effect, respectively. In the analysis to test the E_t/H_t ratio throughout 24 h including all prey species and feeding events, values of E_t and H_t were used as a response variable and an offset, respectively. The explanatory variables and the random intercept effect were the WW and identity of the octopus, respectively.

It can be expected that the prey choice of each juvenile octopus changes depending on its predatory capacity, manifested as: (1) the number of individuals of each prey species consumed (chosen) by the octopus; (2) the time spent in consuming each prey species; and (3) the energy it obtained from each prey species. To investigate (1), the change in proportion of prey species, a binomial GLMM (logit link) was used, in which the proportion of each type of prey among the total number of prey animals (N_t) consumed was a response variable (i.e., N_b/N_t , N_g/N_t and N_m/N_t for bivalves, gastropods, and mysids, respectively). The explanatory variables were octopus WW, prey species (as a categorical fixed factor), and their interaction. To assess (2), the change in proportion of time spent consuming a particular prey species, a binomial GLMM was applied using the proportion of H for the prey species among the total H_t over 24 h as a response variable (i.e., H_b/H_t , H_g/H_t and H_m/H_t for bivalves, gastropods, and mysids, respectively). The explanatory variables were octopus WW, prey species (as a categorical fixed factor), and their interaction. The binomial GLMM was also applied to estimate (3) the change in energy dependence of the octopus on each prey species. In this analysis, the proportion of E obtained from a certain prey species by the octopus among the total E_t over 24 h was used as a response variable (i.e., E_b/E_t , E_g/E_t and E_m/E_t for bivalves, gastropods, and mysids, respectively). The explanatory variables were octopus WW, prey species (as a categorical fixed factor), and their interaction. In these GLMMs, identity of the octopus was used as a random intercept, and the statistical significance of each explanatory variable was evaluated using the *Anova* function (type II, Wald χ^2 tests) in the 'car' package.

The rank order in which each prey organism was consumed by each octopus can also be interpreted as a consequence of prey choice by the octopus. And the choice may depend on the criteria E (energy maximiser: how much energy can be obtained from a prey animal), H (time-minimizer: how fast the octopus can consume a prey animal), or E/H (rate maximiser: how fast energy can be obtained from a prey animal), following the assumptions of foraging theory (Pyke et al. 1977; Stephens and Krebs 1986; Stefens et al. 2007). For instance, if the octopus shows a higher priority for E/H , the E/H of a feeding event is expected to decrease among the animals subsequently preyed upon. This is because the octopus is expected to choose first the prey with the highest E/H . To test whether or not the juveniles followed these three criteria, the Gamma GLMM was used with the log link function. In this analysis, three criteria H_i , E_i and E_i/H_i were used as separate response variables in three GLMMs, where H_i and E_i were handling time and energy of a prey animal selected i -th by an octopus, respectively. The explanatory variables were the order (i) of the prey organism consumed and the octopus WW. Taking into account the effect of ontogenetic development of predatory capacity on octopus prey choice (see Results section, Figs. 2, 3), this analysis was applied separately to early juveniles of WW < 2.5 g (mainly younger than 25 d) and grown juveniles of WW > 2.5 g (mainly 30 – 50 d). The boundary (2.5 g WW) between early and grown juveniles was chosen to bisect the test range of WW of juveniles (0.08 – 5.22 g), to secure enough data for the analyses of these two stages.

Results

Ontogenetic development of predatory capacity

Juvenile *A. fangsiao* preyed on all three prey species. Although the force required to open the valves increased with the size of the bivalve (Table S2, Fig S2a), the mean and maximum sizes of bivalves consumed by octopuses were significantly larger as the octopuses grew (Table S3, Fig. 2a). Newly hatched juveniles could eat bivalves of SL < 5.3 mm. Beyond a WW of 1.5 g, they consumed bivalves of SL > 10 mm. Of the 130 recorded bivalve feeding events, the opening of valves by use of drilling was observed only once (for the largest octopus, 5.2 g WW) on a relatively large bivalve of SL 9.4 mm. In all other instances, feeding commenced after the octopus opened the bivalve by pulling the valves apart (Supplementary Video S1). The ontogenetic development of shell-opening ability (force) by the octopus was estimated based on the relationship between the maximum size of bivalves consumed and the force required to open them by pulling (Fig. 2b). Newly hatched juveniles of 0.1g WW were estimated to exert 0.37 N, increasing to 0.7, 0.9, 1.2, and 1.7 N at, respectively, 0.5, 1.0, 2.0, and 5.0 g WW.

In the gastropod size range 4.7 – 9.6 mm SH, there was no significant correlation between shell size and force required to detach them (Table S2, Fig. S2b): the mean force of detachment was calculated to be 0.12 ± 0.08 N, regardless of gastropod size. Immediately after hatching, the octopuses were able to consume a gastropod of 5.7 mm SH (Fig. 2c) but thereafter the mean and maximum sizes of gastropods consumed increased significantly with octopus WW (Table S3). After reaching 0.6 g WW, the octopuses were able to eat gastropods of > 7 mm SH (Supplementary Video S2). Drilling of gastropod shells was never observed. When the octopuses attempted to catch a mysid, they often failed (Fig. 2d, Supplementary Video S3). The successful capture rate was low at first (< 3% for octopuses of 0.3 g WW) but increased with octopus WW (Table S2). The estimated relationship using GLM describing the success rate rose to 29% for an octopus of 5.0 g WW.

Regarding the handling time, H_t spent to consume prey organisms, the octopuses required 195 ± 103 min per 24 h to consume prey organisms throughout the experimental period (Fig. S3a), which is the equivalent of 13.6% of a day. The growth of the octopuses (increase in WW) did not significantly affect the H_t (Table S4). When octopuses consumed bivalves, H_b to consume individual prey decreased significantly with increasing octopus WW, but it increased with increasing bivalve SL (Table S5, Fig. 3a). For gastropods, too, H_g decreased as the octopus grew, but gastropod size did not affect H_g (Fig. 3b). Likewise, if the octopus preyed on mysids, H_m decreased with increasing octopus WW (Fig. 3c). These results indicate that, as they grew, the octopuses consumed prey organisms faster. In addition, it is noteworthy that, for all prey species, the rate of shortening of H was faster in early juvenile octopuses of < 2.5 g WW than in grown juveniles. Comparing prey species, octopuses required more time to consume a gastropod than a bivalve or a mysid.

Bivalves, gastropods, and mysids had a similar energy content but crude protein content differed markedly, and only gastropods contained carbohydrate (Table S1). The mean energy intake, E_t by an octopus for 24 h was 69.8 ± 26.7 J on the day of hatching (Fig. S3b), and thereafter increased significantly with octopus WW (Table S4). The rate of energy intake per unit time (E/H) from an individual prey animal increased significantly with octopus WW for all prey species (Table S6, Fig. 4a – c). Although

the E/H values were lower than 50 mJ s^{-1} for most newly hatched octopuses for all prey species, the increase of E/H with octopus growth differed among prey species. According to the estimation using the GLMM, the E/H values for an octopus of 2 g WW were 195, 62, and 52 mJ s^{-1} when consuming, respectively, a bivalve (E_b/H_b), a gastropod (E_g/H_g), or a mysid (E_m/H_m). These values increased, respectively, to 425, 89, and 71 mJ s^{-1} for an octopus of 4 g WW. Thus, E/H values rose faster for bivalves compared with both gastropods and mysids. Values of E_t/H_t for all feeding events during the test period (24 h) also increased significantly with increasing octopus WW (Table S6, Fig. 4d).

Choice of prey species, time allocation, and energy dependence

The proportion of a certain prey species among the total number of prey organisms consumed in 24 h by an octopus varied (Fig. 5a – c), indicating that there was a clear prey choice by the octopus. This choice was less clear for small octopuses of $< 1.0 \text{ g WW}$ due to the small number of prey individuals consumed but thereafter, a relatively high proportion of mysids (N_m/N_t , 40%) was observed. The proportions of bivalves (N_b/N_t) and gastropods (N_g/N_t) showed contrasting changes with octopus growth: bivalves were less frequently eaten by small octopuses but the proportion N_b/N_t increased with increasing octopus WW; while gastropods were at first consumed more frequently ($> 40\%$) but the proportion N_g/N_t decreased as the octopuses grew. The proportion of bivalve prey exceeded that of gastropods when the juveniles reached 1.9 g WW. Reflecting these trends, a statistically significant effect on prey choice was detected for the prey species and in the interaction of prey species with octopus WW (Table S7, Fig. 5d).

The handling time, H , allocated to consume each prey species in the H_t for 24 h differed among prey species (Fig. 6a – c). The early juvenile octopuses spent more time in consuming gastropods (H_g/H_t) at around 70%, which increased slightly with increasing WW of the octopus. This is obviously longer compared to those of bivalves (H_b/H_t) and mysids (H_m/H_t), which were typically $< 20\%$. Thus, octopuses allocated H differently for each prey species, and it changed with increasing octopus WW (Table S7, Fig. 6d).

The energy dependence of octopus on a certain prey species changed along with their growth depending on the prey species (Table S7, Fig. 7a – c). During the period when octopus WW was less than 2.7 g, the early juveniles depended largely on gastropods (E_g/E_t) to obtain energy more than 50% of the source. However, the degree of dependence on the gastropod decreased linearly with increasing octopus WW. In contrast, the dependence on bivalves (E_b/E_t) was low at first but thereafter it increased as the octopus grew and exceeded that on gastropods at WW 3.5 g (Fig. 7d). The dependence on mysids (E_m/E_t) was always relatively low, $< 20\%$. Thus, the octopuses shifted their energy source by changing their choice of prey species and size as they grew.

Ranking of prey choice

In early juveniles of WW < 2.5 g, mainly younger than 25 d after hatching, the proportion of gastropods was highest among the three types of prey organism from the first to third 'meal' consumed, and then it decreased among the animals subsequently preyed upon (Fig. 8a). At the fourth and fifth meal, the proportion was similar among the three prey species, and the proportion of mysids was higher than that of bivalves and gastropods from the sixth meal onward. These results indicate that the early juveniles chose to eat gastropods more preferentially than bivalves and mysids, although they could consume any of the available prey species. The GLMM analyses revealed that E_i was significantly high for prey items chosen earlier (Table 1, Fig. 8c). There were no significant relationships between order of prey consumed and H_i or E_i/H_i (Fig. 8e, g). This implies that prey choice by the early juveniles did not shorten H , and thereby increase E/H , but increased E directly.

In grown juveniles of 2.5 – 5.2 g WW, aged 30 – 50 d, the proportion of mysids was highest among the first to forth meal consumed, but decreased with increasing number of prey items eaten (Fig. 8b). For the first and second prey animals consumed, the proportion of bivalves was second highest and that of gastropods was lowest. From the fifth item onward, the proportions of all prey species fell within the range of 21 – 43%. These results indicate that older juveniles preferred the prey species in the order mysids > bivalves > gastropods. Regarding the relationships between E_i , H_i or E_i/H_i , and the order (i) in which prey was consumed, no significant relationship was found between E_i and order of prey consumed (Table 1, Fig. 8d), but H_i was significantly shorter for prey items chosen earlier (Fig. 8f), indicating that the octopuses tended to first choose prey that they could consume quickly. The relationship between the E_i/H_i and order of prey consumed was also statistically significant. E_i/H_i tended to decrease down the rank of prey consumption (Fig. 8h), implying that the octopuses preferred to consume prey from which they could obtain energy more quickly. In short, the order of prey choice changed as the octopuses grew. Early juveniles chose prey organisms to increase E since they showed a high preference for gastropods, but older juveniles chose to shorten H and thereby increase E/H by selecting mysids and bivalves earlier.

Table 1

Coefficient estimates with standard error (SE) for the GLMM to examine the effects of order of prey consumed and wet weight (WW, g) of *Amphioctopus fangsiao* on uptake energy (E_i), handling time (H_i), and rate of energy uptake per unit time (E_i/H_i) from individual prey organisms

Response variable	Explanatory variable	Coefficient	SE	t	P
Octopus size < 2.5g WW					
Energy (J)	Intercept	3.914	0.111	35.4	< 0.0001
	Order of prey consumed	-0.035	0.016	-2.26	0.0240
	Octopus WW	0.318	0.076	4.17	< 0.0001
Handling time (s)	Intercept	7.013	0.145	48.4	< 0.0001
	Order of prey consumed	-0.0030	0.0201	-0.15	0.8810
	Octopus WW	-0.501	0.102	-4.91	< 0.0001
Energy/Handling time ($J s^{-1}$)	Intercept	-2.428	0.134	-18.1	< 0.0001
	Order of prey consumed	0.0014	0.0180	0.078	0.9390
	Octopus WW	0.777	0.080	9.67	< 0.0001
Octopus size > 2.5g WW					
Energy (J)	Intercept	3.847	0.393	9.78	< 0.0001
	Order of prey consumed	0.017	0.015	1.11	0.2680
	Octopus WW	-0.144	0.286	-0.50	0.6140
Handling time (s)	Intercept	6.242	0.596	10.5	< 0.0001
	Order of prey consumed	0.064	0.023	2.74	0.0061
	Octopus WW	0.160	0.412	-0.39	0.6982
Energy/Handling time ($J s^{-1}$)	Intercept	-2.550	0.465	-5.49	< 0.0001

Response variable	Explanatory variable	Coefficient	SE	t	P
	Order of prey consumed	-0.040	0.015	-2.64	0.0084
	Octopus WW	1.106	0.352	3.15	0.0017

Log-transformed WW was used in the GLMMs (generalized linear mixed-effects models)

Discussion

Newly hatched *A. fangsiao* juveniles consumed all three prey species (bivalves, gastropods, and mysids) while showing predatory behavior using their arms and suckers immediately after hatching, such as pulling open bivalve shells, detaching gastropods from the substrate, and capturing swimming mysids immediately after hatching. It is well known that benthic octopuses prey on various animals, including molluscs, crustaceans, polychaetes, and fishes, so they are recognized as generalist predators (Ambrose 1984; Iribarne et al. 1991; Anderson et al. 1999, 2008; Mather 1991; Smith 2003; Mather et al. 2012, 2014; Scheel and Anderson 2012). The findings of the present study suggest that *A. fangsiao* is already a generalist predator at hatching. Nevertheless, the success rate of newly hatched *A. fangsiao* in capturing mysids was less than 2%. Taking into account that the success rate might be further lowered in the natural habitat, where mysids can swim away freely in the absence of a limiting experimental arena, the predatory capacity of *A. fangsiao* on highly mobile swimming crustaceans appears to be particularly poor during their early life just after hatching. Furthermore, the size of bivalves (SL) and gastropods (SH) consumed by newly hatched *A. fangsiao* was limited to 6 mm, implying that there are a number of limits on the type of prey organisms available to early juveniles. Although the feeding habits of *A. fangsiao* juveniles in the natural habitat are poorly known, small-sized molluscs and crustaceans of low motility appear to be their main sources of food.

When the juveniles preyed on the bivalves and gastropods, drilling was observed only for the largest juvenile (5.2 g WW) on bivalves and was never applied to a gastropod shell. It has been reported that adult benthic octopuses can penetrate both bivalve and gastropod shells (Ambrose and Nelson 1983; Fee et al. 2023; Hamada 1974; Markaida 2023; Steer and Semmens 2003; Wodinski 1969), and that *A. fangsiao* > 7 g WW can open bivalve shells by drilling in addition to pulling them apart (Ebisawa et al. 2011). These observations suggest that drilling is impractical for consumption of shelled molluscs by early *A. fangsiao* juveniles smaller than 5 g WW.

It was estimated that to open bivalve shells of 5 mm SL by pulling requires a force of 0.46 N, increasing with larger SL, but only 0.12 N was required to detach a gastropod, regardless of SH. Thus, *A. fangsiao* apparently had easier access to the flesh of gastropods than that of bivalves. Presumably, for this reason early juveniles tended to prefer gastropods rather than bivalves, and they depended largely on gastropods as their energy source. Smith (2003) reported that the proportion of winkles (small gastropods) was larger among the prey items of small octopuses of species *O. vulgaris* compared to

those of medium and large octopuses in the natural habitat, which also consumed a more diverse selection of prey organisms. Although the species size and size range (0 – 300 g WW, 'small' octopuses) of Smith (2003) differed from the present study (0.1 – 5.2 g WW, juveniles), the similarities in the results of these two studies suggest that the availability of prey species to the growing animals changes during growth, as their predatory capacity improves.

Gastropods seem to be a good prey organism for early juvenile octopuses with developing predatory capacity because of their slow motility and easy detachment from the substrate. However, gastropods have the disadvantage of a relatively long handling time (H_g) compared to that for bivalves (H_b) and mysids (H_m). The relatively long H required to consume a gastropod is presumably due to the defence of the gastropod, using its operculum, and the complexity of gaining full access to the animal within the spiral structure of its shell. Thus, early juveniles devote much time to obtaining the energy available in a gastropod. That is, to obtain E requires a trade-off with H .

It has been considered that octopuses are at a high risk of predation during foraging away from their den or other refuge (Mather et al. 2014; Hanlon and Messenger 2018). Therefore, they are considered to choose to minimize their foraging time in the natural habitat to reduce predation risk (Mather and O'Dor 1991; Leite et al. 2009). In general, predation risk tends to be higher in younger animals in a size dependent manner (Anderson 1988; Urban 2007). Thus, juvenile octopuses appear to be more vulnerable to predation than adults due to their small body size. The findings of the present study suggest that predation risk is further elevated for early juveniles of *A. fangsiao*, not only due to the size-dependent predation risk but also to the longer H required for prey consumption (risky feeding) arising from their underdeveloped predatory capacity and consequently restricted range of available prey.

As the juvenile octopuses grew, they were able to consume larger bivalves and gastropods, and the success rate of capturing mysids also increased with increasing octopus WW. Further, the time required to consume prey organisms shortened as they grew, particularly in early juveniles. These developments in predatory capacity are considered to be achieved by gaining in physical strength and consumption efficiency. In particular, obvious enlargement of the size of bivalves consumed and distinct reduction of H_b required to consume them imply that the physical capabilities of the arms and suckers improved with growth, because they opened bivalve shells mostly by pulling. This is in agreement with reports that the size of mussels consumed by *O. vulgaris* is correlated with octopus size (Smale and Buchan 1981; McQuaild 1994). The *A. fangsiao* juveniles could attain faster rates of energy intake (E/H) when consuming larger bivalves compared to gastropods and mysids, presumably due to easy access to the flesh after pulling them open (see Fig. 4). As their predatory capacity on bivalves developed, their main energy source shifted gradually from gastropods to bivalves (see Fig. 7). This implies that the octopuses change their prey preference as their predatory capacity develops, to obtain more energy in a shorter time. Indeed, the total energy intake for 24 h (E_t) increased as the octopus grew, though the total time to consume prey within a day (H_t) did not increase with growth. In the present study, the juveniles were offered varying sizes of bivalves to test their predatory capacity and prey choice. This might restrict their prey choice, particularly in grown juveniles, because they were unable to choose their preferred prey

continuously. If the grown juveniles could choose bivalves of any size freely, presumably they could further shorten H_t and increase E_t/H_t .

It can be assumed that, as the predatory capacity of an octopus develops, the choice and size of potential prey species expand: Smith (2003) reported that the prey species of *O. vulgaris* became more diverse as the octopuses grew in size in the natural habitat. In this context, the *A. fangsiao* juveniles probably change their prey choice criteria as they grow. In the present study, early and grown juveniles were tested to find out whether or not they followed decision criteria such as time-minimization (reducing H), energy maximization (increasing E), or rate maximization (increasing E/H) in choosing their prey (Stephens and Krebs 1986; Stefens et al. 2007). The range and size of prey species were limited in our experimental design, and it is likely that early juveniles can consume a more diverse selection of prey organisms in the natural habitat. Despite these limitations, the early juveniles exhibited significant prey preference tendencies as the order in which they consumed prey chosen from the range of available prey organisms, which can be interpreted as an output of the decision criteria of prey choice. Also, the early juveniles (< 2.5 g WW, approximately first month of life) tended to prefer gastropods, prey containing more energy (E_i). However, H_i and E_i/H_i had no apparent relation to the order of prey choice of the early juveniles, indicating that early juvenile *A. fangsiao* give priority to maximizing the energy (E) in their prey choice (i.e. they are “energy-maximizers”). Grown juveniles (> 2.5 g WW, from the second month of the life) preferred prey organisms for which they could shorten H_i and improve E_i/H_i by choosing mysids and larger bivalves. This tendency suggests that they are “time-reducing rate-maximizers” giving priority to gaining energy intake per unit time by reducing time spent in prey consumption.

Why do they change the criteria of prey choice depending on growth? Field surveys have revealed that, although benthic octopuses are generalist predators, they exhibit selectivity of prey choice at an individual level (Ambrose 1984; Iribarne et al. 1991; Vincent et al. 1998; Anderson et al. 1999, 2008; Mather 1991; Smith 2003; Mather et al. 2012, 2014; Scheel and Anderson 2012). The decision criteria of prey choice in the natural habitat are difficult to investigate but attentive observations of their foraging behavior have suggested that benthic octopuses give priority to shortening their time of foraging to reduce predation risk (i.e., time-minimizers; Mather and O’Dor 1991; Leite et al. 2009). Several laboratory studies have supported this hypothesis by demonstrating that octopuses show prey choice or preference for the method of prey consumption (drilling or pulling to open bivalve shells) to shorten H or maximise E/H (McQuaid 1994; Ebisawa et al. 2011; Song et al. 2019). This hypothesis seems to be supported by our finding that grown *A. fangsiao* juveniles exhibit prey choice minimizing H_i and maximizing E_i/H_i but is inconsistent with the behavior of early juveniles which choose prey to maximize E_i .

The reason for this apparent inconsistency may be associated with the development of predatory capacity. Early juveniles appear to be more susceptible to predation risk due to their small body size and longer H to consume prey, as they are younger. But to put it the other way around, predation risk decreases as their growth accelerates because they can shorten H while going quickly through the period of high risk. So, there is an unavoidable trade-off between the risks of feeding and the intake of energy for growth (Lankford et al. 2001; Biro et al. 2006; Urban 2007). It is noteworthy that, in the present

study, the rate of development of predatory capacity was faster, particularly during the early juvenile period (see Fig. 2, 3). In such a situation, the investment in growth seems to be an effective way to reduce predation risk during the vulnerable early-juvenile period. In addition, growth may contribute to the avoidance of (reducing the risk of) starvation, because the available range of prey choice expands as the predatory capacity of the octopus develops. After reaching the 'grown' juvenile, the decision criteria of prey choice switches to balance predation risk and growth, by giving priority to shortening H and increasing E/H . The development of the predatory capacity of the juveniles, as well as the increased juvenile antipredation repertoire (including camouflage, mimicry, and deimatic behavior), may be associated with the timing of preference switching. This switchover strategy (firstly give priority to growth, then subsequently to balance predation risks and growth) seems to be a good compromise to survive the period of high risks of predation and starvation during early life.

Declarations

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Data availability

Data will be made available on request.

Conflict of interest

The authors have no conflicts of interest directly relevant to the content of this article.

Ethical approval

This study was approved by the Committee on the Ethics of Animal Experiments at Tokyo University of Marine Science and Technology (permission number: R2-S1), where the experiments were carried out.

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Figures

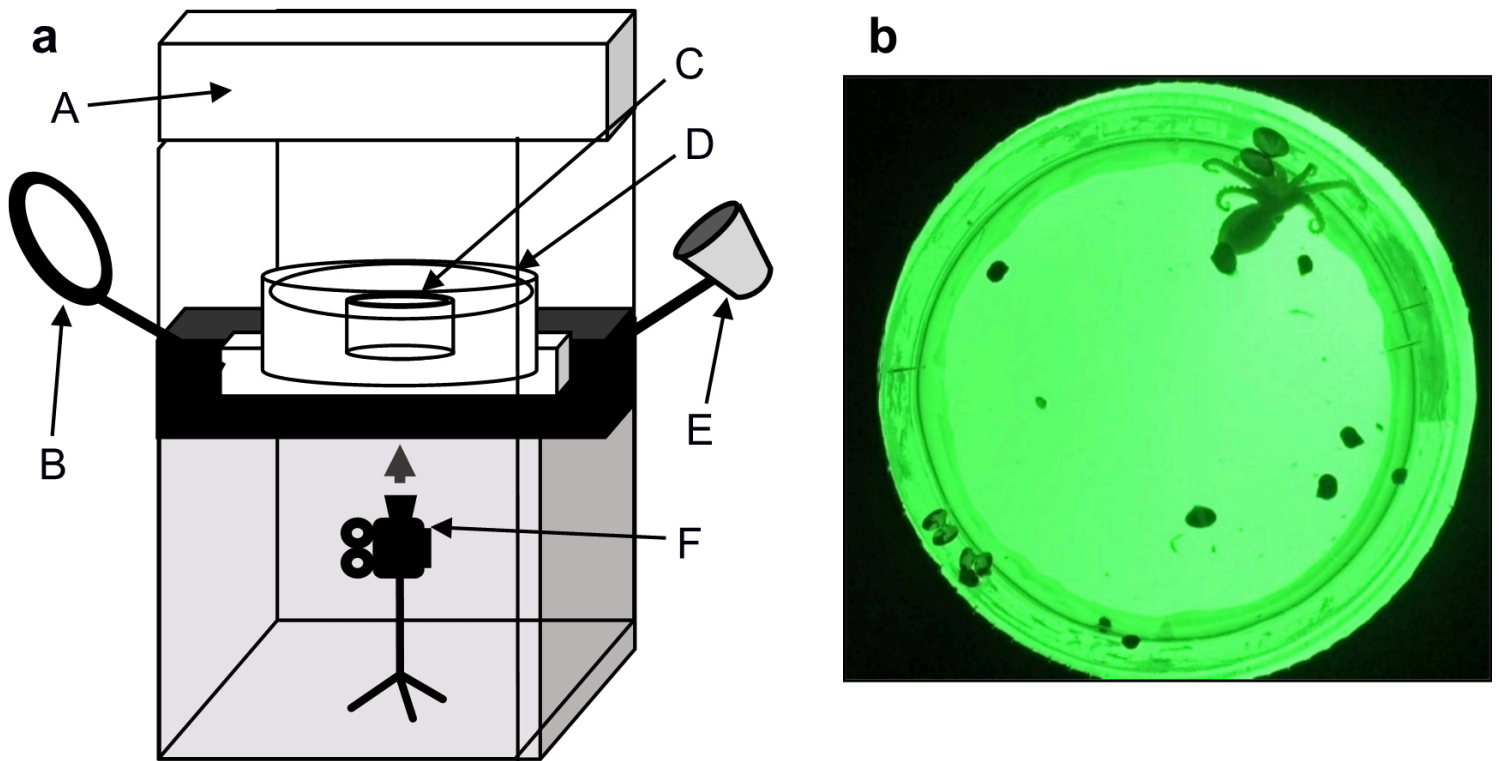


Figure 1

(a) Diagram of the experimental arena and video recording system. (b) Capture image from a video recording of the experimental arena. (A) white PVC board, (B) LED light, (C) inner acrylic arena, (D) outer acrylic container, (E) infrared LED light, and (F) video camera

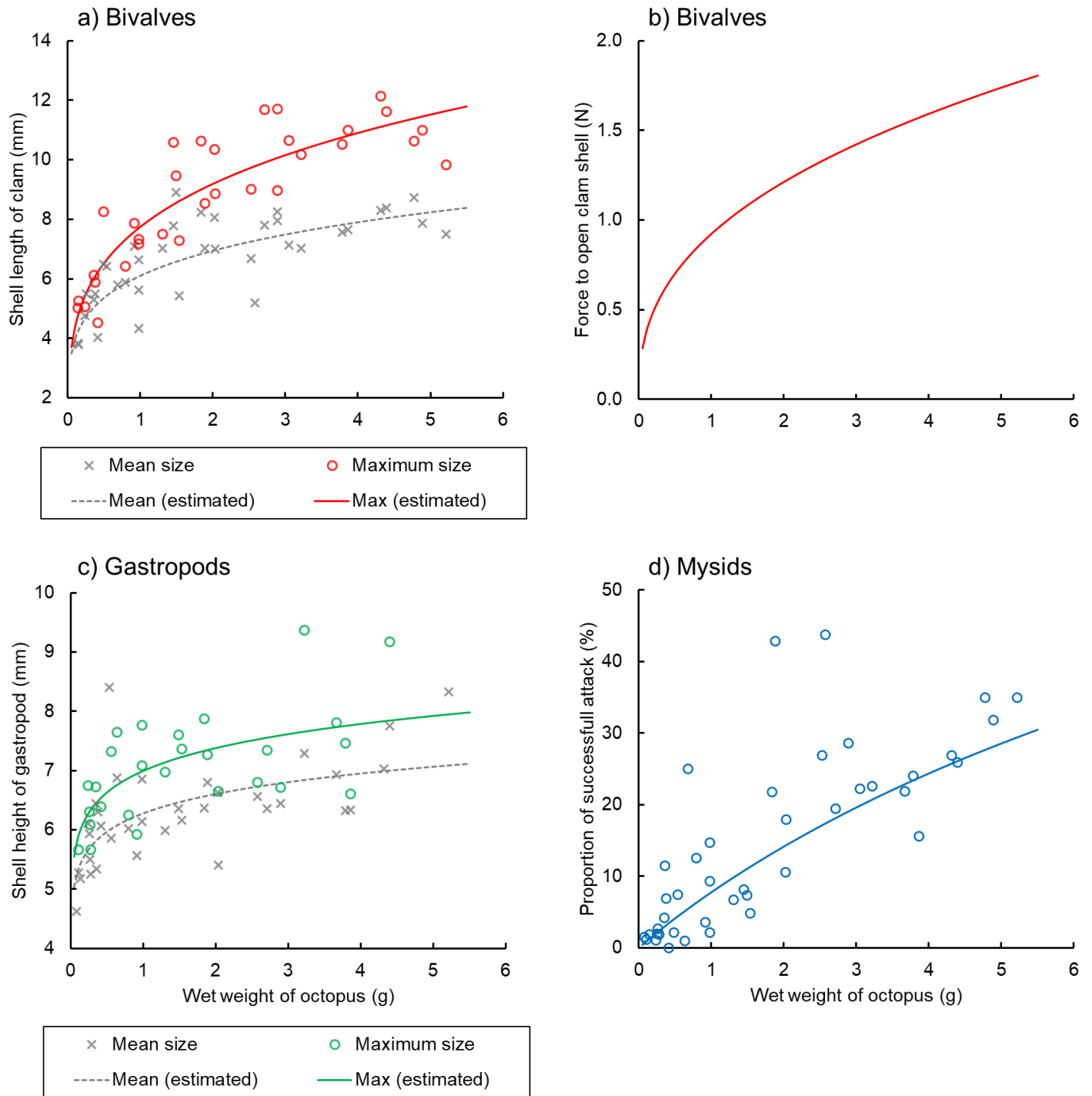


Figure 2

(a) Size relationship between *Amphioctopus fangsiao* juveniles and bivalves consumed. (b) Estimated ontogenetic change in force exerted by octopuses to open bivalve shells. (c) Size relationship between octopuses and gastropods consumed. (d) Proportion of successful attacks by octopuses to capture mysids. Solid and broken lines are estimates from LMMs and a GLMM

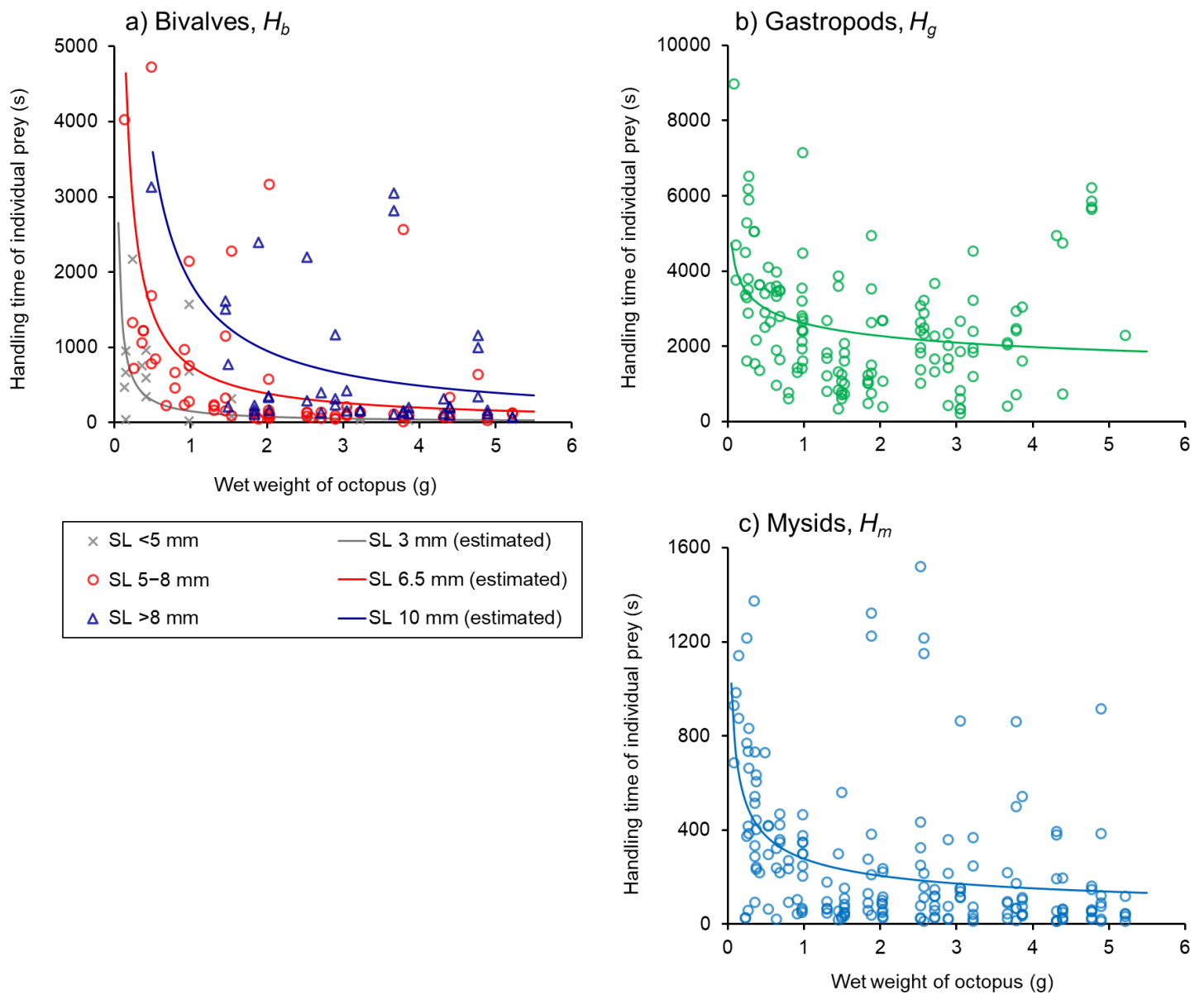


Figure 3

Changes in handling time to consume **(a)** a bivalve H_b , **(b)** a gastropod H_g , and **(c)** a mysid H_m by *Amphioctopus fangsiao* juveniles. Solid and broken lines are probability curves where $P < 0.05$, estimated using GLMMs

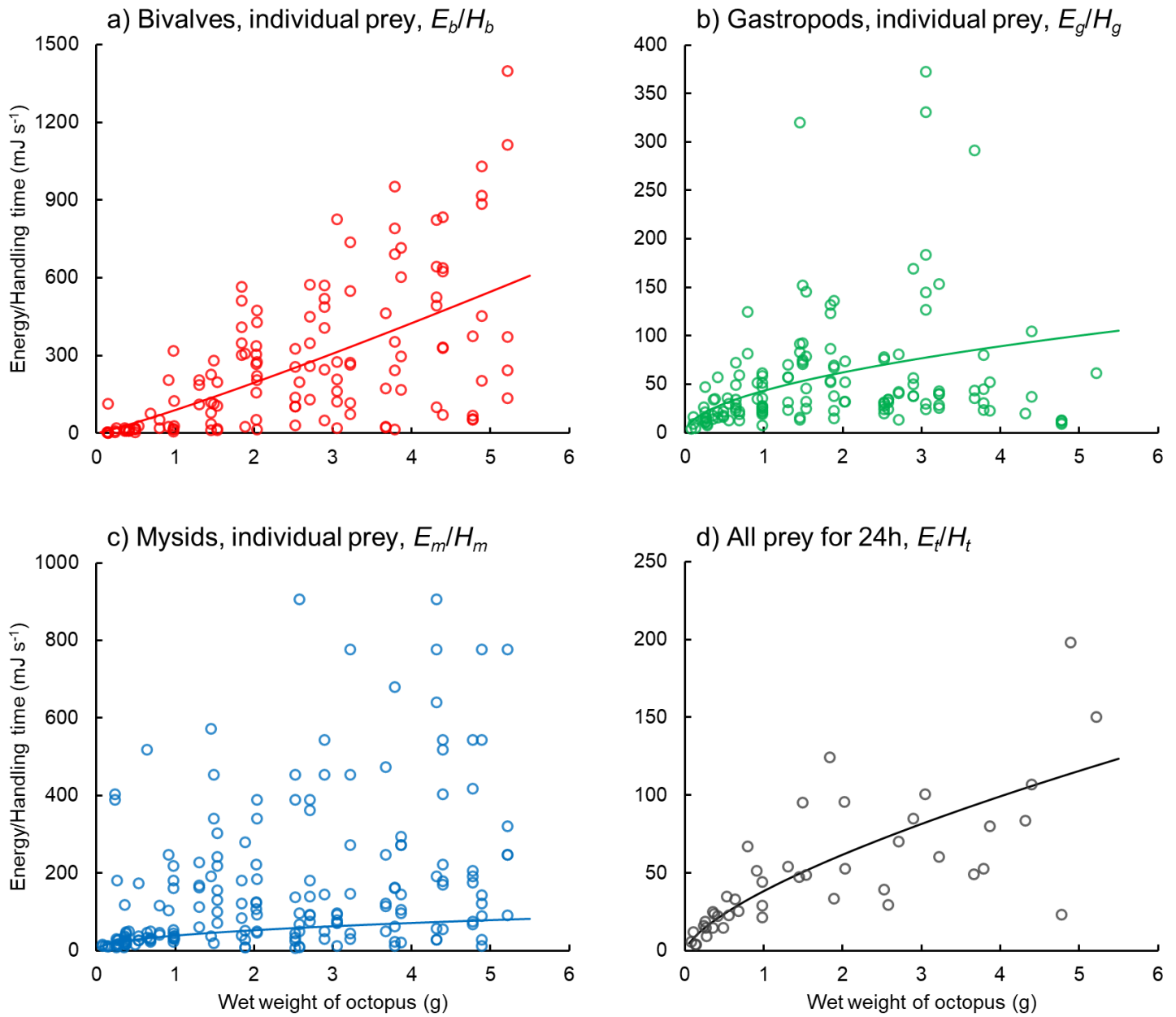


Figure 4

Changes in rate of energy intake per unit time when feeding on **(a)** a bivalve E_b/H_b , **(b)** a gastropod E_g/H_g , **(c)** a mysid E_m/H_m , and **(d)** all prey items E_t/H_t (per 24 h) by *Amphioctopus fangsiao* juveniles. Solid lines represent statistically significant coefficients at $P < 0.05$, estimated using GLMMs

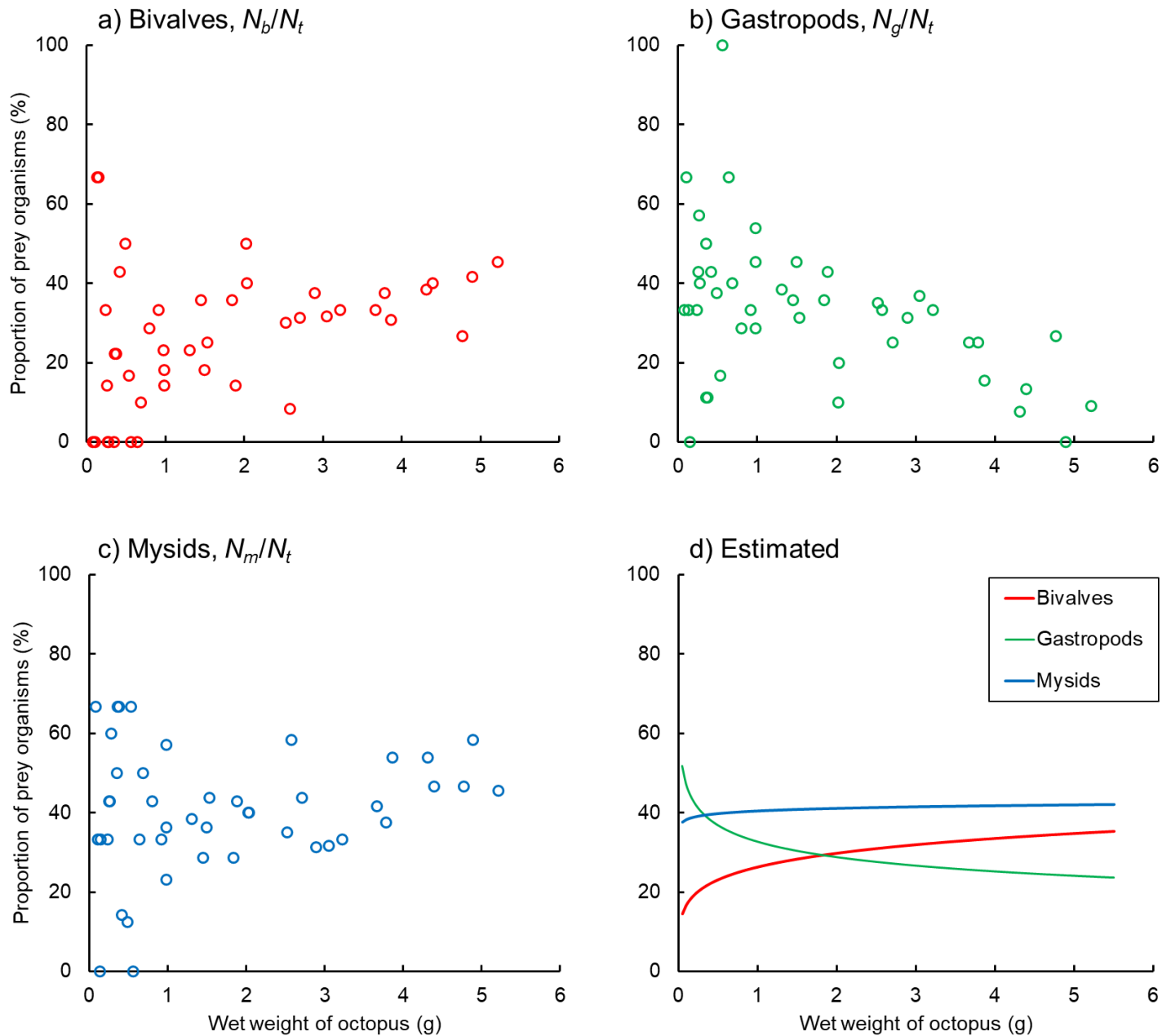


Figure 5

Changes in the proportion of **(a)** bivalves N_b/N_t **(b)** gastropods N_g/N_t and **(c)** mysids N_m/N_t among the total number of prey organisms consumed by *Amphioctopus fangsiao* juveniles per 24 h. **(d)** Curves showing proportions of the different prey species consumed, estimated using GLMMs

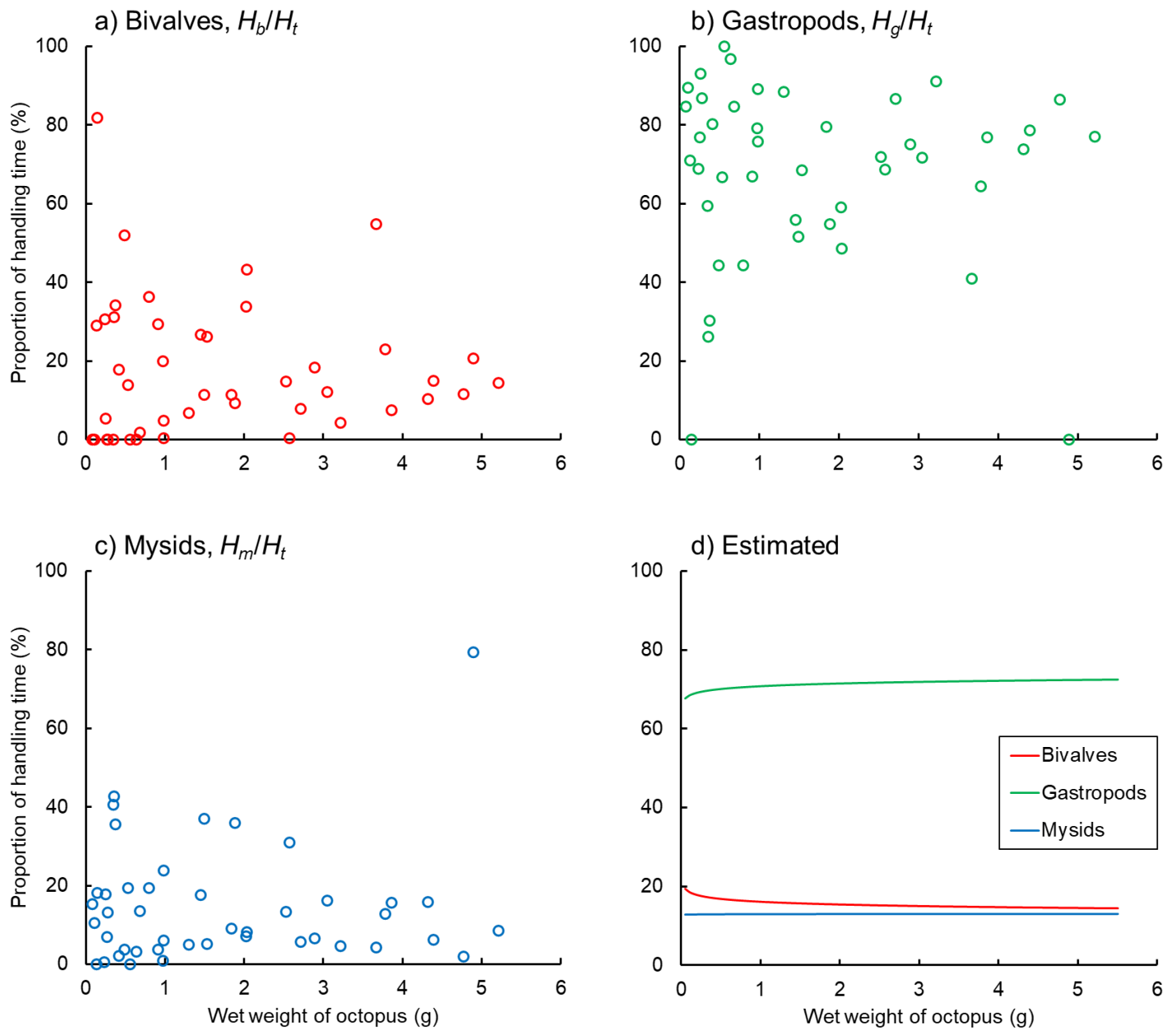


Figure 6

Changes in time to consume **(a)** bivalves H_b/H_t **(b)** gastropods H_g/H_t and **(c)** mysids H_m/H_t as a proportion of the total handling time for all prey organisms consumed by *Amphioctopus fangsiao* juveniles in 24h. **(d)** Curves of proportions of respective prey species in the total handling time of all prey organisms consumed, estimated using GLMMs

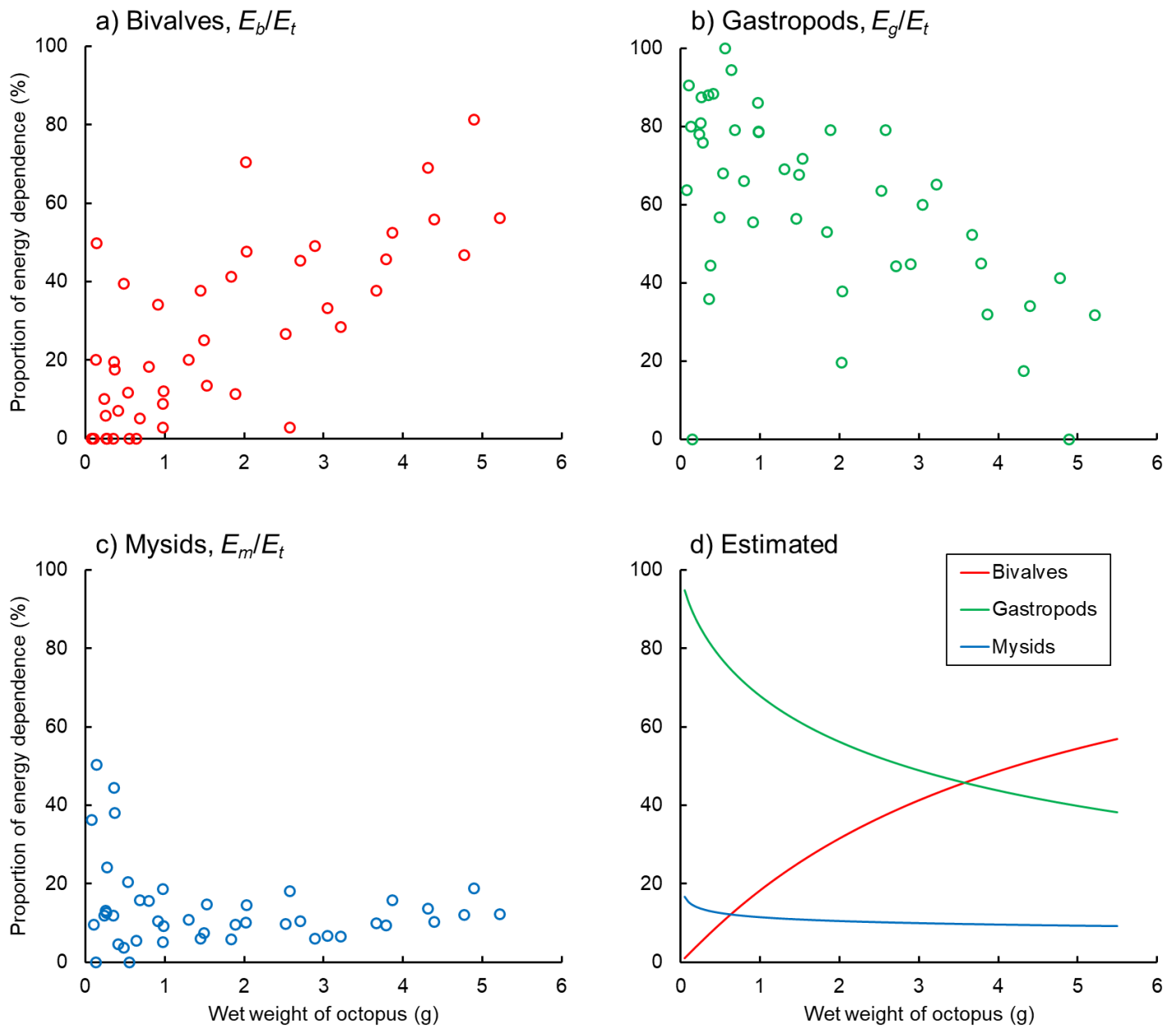


Figure 7

Prey species contribution to energy input from 24 h experimental feeding periods during growth of *Amphioctopus fangsiao* juveniles: (a) bivalves E_b/E_t (b) gastropods E_g/E_t and (c) mysids E_m/E_t (d) Curves summarizing changes in proportion of total energy intake for the three prey species, estimated using GLMMs

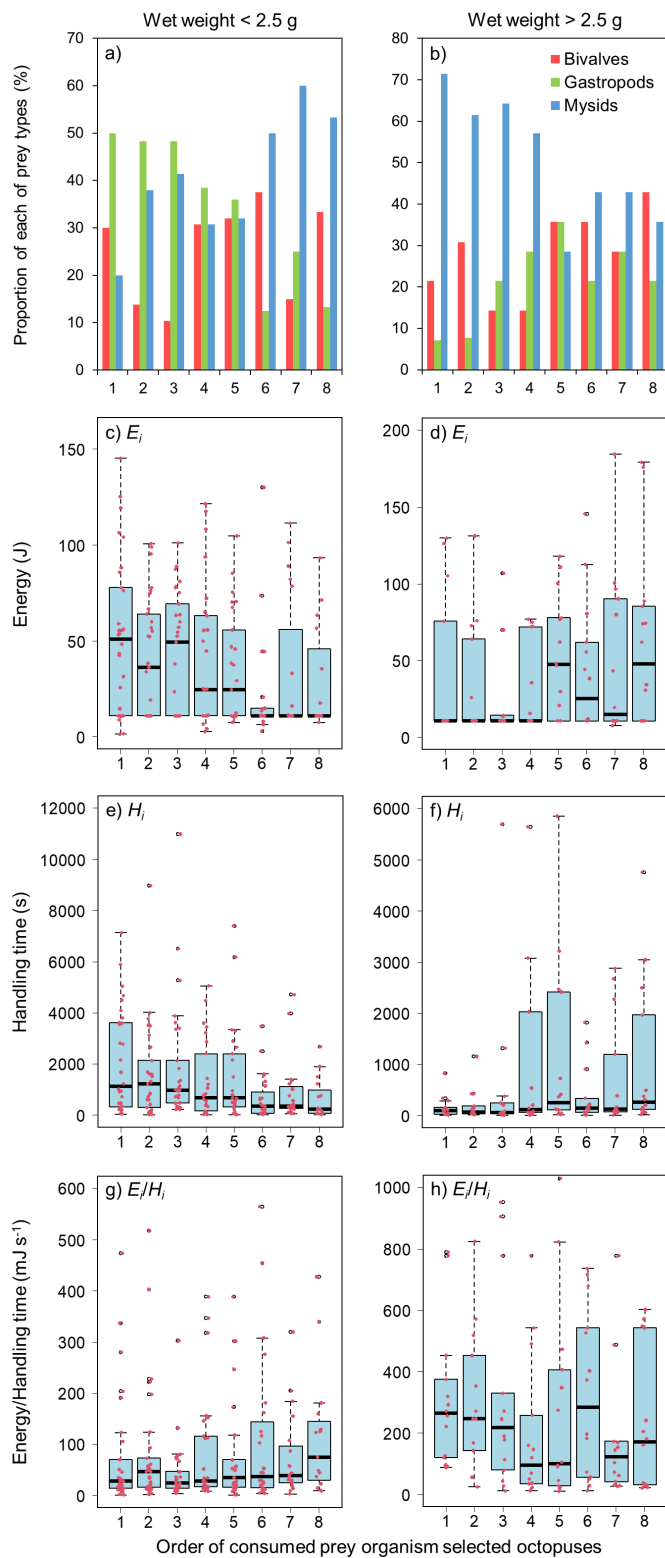


Figure 8

Analysis of selection and handling of prey as consumed by *Amphioctopus fangsiao* of wet weight < 2.5 g (a, c, e, g) and > 2.5 g (b, d, f, h). Numbers along base of each chart denote the ranking (order of selection, i) of first 8 prey items ('meals') selected and consumed by each individual. (a, b) Percentage of each of the three prey items selected by the octopuses at each 'meal' rank. (c, d) Box plots of the energy content E_i (calculated using measured prey size) for each prey item selected at each of the eight ranks,

with all individual data points superimposed. (**e, f**) Box plots of handling times H_i . (**g, h**) Box plots of energy per second of handling time E_i/H_i

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryVideoS1Aged30dbivalve.mp4](#)
- [SupplementaryVideoS2Aged50dgastropod.mp4](#)
- [SupplementaryVideoS3Aged0dmysid.mp4](#)
- [Supplementaryinformation.docx](#)