

Prey selection and foraging behavior of the whelk *Rapana venosa*

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Abstract *Rapana venosa* is one of the most widespread species of muricid gastropod and lives on all types of substrata. Although common in Bohai Sea, relatively little is known about its ecology specially foraging behavior. We examined (1) prey selection by *R. venosa* when offered three prey species, Manila clams *Ruditapes philippinarum*, blue mussels *Mytilus edulis* and oysters *Crassostrea gigas*, (2) relative importance of various behavioral components (i.e., encounter, attack, capture and consumption) to the prey selection and (3) in detail the foraging behaviors of *R. venosa* in the laboratory. Predation rates, prey characteristics (shell strength, energy content per prey, handling time per prey), behavioral components (encounter probabilities, foraging time budget) and major behavioral transition frequencies were analyzed. *R. venosa* consumed more clams per day than mussels and oysters. Predation on clams was the most effective with a consumption rate of 0.75 clams

day⁻¹, although whelks spent only 1.23 % of their time searching for prey. In comparison, 0.54 mussels day⁻¹ were consumed while spending 2.28 % of their time searching, and 0.29 oysters day⁻¹ were consumed occupying 3.08 % of the time. With the preference for clams over mussels and oysters resulted from *R. venosa* active selection, the prey has a higher profitability. The probability of capture upon attack and consumption upon capture, reflected prey shell strength and morphology, was the two most important behavioral components to determine the prey selection for clams. Searching events took place more often when offered oysters than mussels and clams. Therefore, prey species selection by *R. venosa* appears to be determined by differences in prey vulnerability and accessibility, and active selection of prey with the highest profitability. Our results have implications for the provision of protective refuges for species of interest (i.e., oysters), such as in population enhancement operations and bottom aquaculture.

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Introduction

Predation, which plays a key role in structuring ecological communities, is an important ecological process and that many activities of both predator and prey such as assemblage, distribution and population dynamics are often driven by predation. In addition, predation of seeded bivalves often causes mortality and limits the success of culture (Flagg and Malouf 1983; Halary et al. 1994; Barbeau et al. 1996). A good knowledge of predator–prey interactions is essential to understand the predation. How predators choose their diets and hunting efficiency are crucial to our understanding of predator–prey interactions, particularly with regard to potential stabilizing properties (Elner and Hughes 1978).

In the Bohai Sea, *Rapana venosa* is a widely distributed and commercially important species. It inhabits all types of substrata and forages mostly on bivalve prey (Harding and Mann 1999; Savini et al. 2002; Savini and Occhipinti-Ambrogi 2006; Kosyan 2015). *R. venosa* is harvested usually by SCUBA for human consumption. As local demand for sea products has increased, *R. venosa* is being considered as a potential candidate for molluscan aquaculture, although it has been colonizing aquatic ecosystems as an invader in the other ocean, like the Adriatic Sea (Ghisotti 1974), the Aegean Sea (Koutsoubas and Voultsiadou-Koukoura 1991) and the American Atlantic Sea/Chesapeake Bay (Harding and Mann 1999). Although it is one of the most common gastropods in the Bohai Sea, relatively little is known about its ecology. As the important fishery resource, the Manila clam *Ruditapes philippinarum* and the oyster *Crassostrea gigas* have become two of four main aquacultured shellfish in China. However, during the enhancement and culture process, predation by *R. venosa* is usually a major constraint to the production. Consequently, providing the target bivalve with a refuge from predation has been considered. The presence of an alternative prey species may provide such a refuge, as predator may choose the alternative prey and consume fewer target bivalve. Mussels *Mytilus edulis* are being considered as the alternative prey, since they are less commercially important. Therefore, it is urgent to know predation of *R. venosa* when offered less commercial prey and commercial prey individually or simultaneously.

Selection of prey by predators could be influenced by many factors, including detectability, accessibility, easy of capture, energy content of prey, time required to handle the prey and time required for non-predatory behavior (Hughes and Dunkin 1984). According to optimal foraging theory, predators actively select specific prey to maximize the net rate of energy intake per unit foraging time (Pyke et al. 1977; Hughes 1980). Underwood et al. (2004) argued that descriptions of diets of predators as the only evidence about preference are not enough and the existence of a preference requires (and implies) an outcome of behavior by an organism. A simple concept, termed the predation cycle, can be typically characterized predation events as a series of behaviors that include searching for, attacking, capturing and consuming prey (Holling 1966; O'Brien 1979; Barbeau and Scheibling 1994). Based on this cycle, predation rates are determined by a number of behavioral characteristics: time budget, encounter rate between the predator and prey, the probability of attack upon encounter, the probability of capture upon attack and the probability of consumption upon capture (Nadeau et al. 2009). Dissection of a predation event into its component parts provides direct information about the mechanisms underlying patterns of prey selection and clarifies the interactions between

predator and prey (O'Brien 1979; Cooper et al. 1985). For example, the encounter rate between the predator and prey and searching time budget could reflect the detectability of predator and the ability for prey to escape. The probability of consumption upon capture and handling time budget may reflect the accessibility, profitability and vulnerability of prey to predator. Until recently, many studies of the predation cycle have focused on crustaceans and echinoderms (Barbeau and Scheibling 1994; Mistri 2004; Wong et al. 2010; Wong 2013), but rarely on gastropods. In addition, ethograms could examine sequences in the behaviors of predators, which include the comprehensive descriptions of the characteristic behavior and clearly show the behavior transitions (Himmelman et al. 2005; Nadeau et al. 2009).

We predicted that *R. venosa* would select a particular prey species, since clams, mussels and oysters would differ in prey characteristics (shell strength, energy content and profitability). Specifically, we predicted that *R. venosa* would active select clams over mussels and oysters, resulting in clams being highest in profitability and oysters being lowest. We predicted that encounter rate would not determine the prey selection, since all of three prey species cannot escape when preying by *R. venosa*. We predicted that the encounter behaviors would show different relative importance in determine prey selection. Furthermore, we predicted that the probability of consumption upon capture would be lowest for oysters. Also, handling time would be highest, resulting in oysters being lowest in profitability and highest in shell strength.

Materials and methods

Experimental materials

The experiments were conducted in the Ecology Laboratory of the Institute of Oceanology, Chinese Academy of Sciences, from August to September 2015. All experimental organisms (*R. venosa*: 70–94 mm shell height; *R. philippinarum*: 21–43 mm; *M. edulis*: 19–48 mm; *C. gigas*: 20–46 mm shell length) were collected from the Laizhou Bay, Bohai Sea. *R. venosa* was collected by a SCUBA diver, transported to the laboratory and acclimated in individual glass aquaria for 2 weeks with seawater (26 ± 1 °C; 30 ‰ salinity) sand-filtered at 1 mm. Manila clams *R. philippinarum* and oysters *C. gigas* were collected from the coastal beach and sub-tidal rocks by ourselves, respectively. Blue mussels *M. edulis* were purchased from a local market, and they were collected by a local fisherman. Whelks were fed twice daily with sufficient fresh clams *Scapharca subcrenata* (purchased from a local aquatic market) before being starved for 6 days prior to the experiment, to standardize hunger level and predation experience level on experimental

Table 1 Biological index of animal

(Mean \pm SD) Biological index	<i>R. venosa</i>	<i>R. philippinarum</i>	<i>M. edulis</i>	<i>C. gigas</i>
Shell length (mm)		34.86 \pm 2.01	36.99 \pm 2.44	36.87 \pm 4.92
Shell height (mm)	75 \pm 5.0	23.69 \pm 1.26	21.49 \pm 1.40	22.84 \pm 4.15

Table 2 Experimental design

Experiment system	Prey type	Prey density as# unit ⁻¹	No. of replicate	Duration (days)	Total time observed (h)
RC	Clam	18	4	6	96
RM	Mussel	18	4	6	96
RO	Oyster	18	4	6	96
RCM	Clam + Mussel	9 + 9	4	4	0
RCO	Clam + Oyster	9 + 9	4	4	0
RMO	Mussel + Oyster	9 + 9	4	4	0
RCMO	Clam + Mussel + Oyster	6 + 6 + 6	4	4	0

prey. Only healthy and whelks with intact shells were used. Whelks were measured as the maximum dimension from spire to canal (Table 1). The three prey species were measured for shell length and shell height (Table 1) and held in separate aquaria (50 cm long \times 40 cm wide \times 30 cm high; 60 L) for 1 week with seawater conditions as described above for the whelks. The photoperiod was set to 14 h light: 10 h dark. The water in the aquaria was aerated and changed regularly (>50 % volume every 2 days).

Experimental design

Prey selection, active selection and predator foraging behavior were examined in three-prey, two-prey (choice) and single-prey (no choice) experiments. To examine prey selection, three-prey experiments were designed and individual whelks were offered six clams plus six mussels and six oysters, represented by RCMO (Table 2). This treatment was replicated four times and lasted 4 days. A single *R. venosa* was randomly allocated to a circular experimental aquarium ($r = 0.5$ m, $h = 0.4$ m; 314 L) 24 h prior to starting an experiment, after which the 18 prey were placed in the aquarium. Two additional aquaria containing the three prey types and no predator were used as controls to monitor natural prey mortality in each experiment (over 4 days, two clams and one mussel died).

To detect active selection and examine foraging behavior, two-prey and single-prey experiments were designed. In two-prey experiments, individual whelks were offered three prey-treatment combinations: nine clams plus nine mussels, nine clams plus nine oysters and nine mussels plus nine oysters, represented by RCM, RCO and RMO (Table 2). Each treatment was replicated four times and lasted 4 days. A single *R. venosa* was randomly allocated to a circular experimental aquarium 24 h prior to starting

an experiment, after which the 18 prey were placed in the aquarium. Two additional aquaria containing the two prey types and no predator were used as controls to monitor natural prey mortality in each experiment (over 4 days, one clam died in the experiment with nine clams plus nine mussels). In single-prey experiment, a video camera was used to record *R. venosa* behavior. There were three treatment combinations: the predator species with one of three prey species (clams, mussels or oysters), represented by RC, RM and RO, respectively (Table 2). Each treatment combination was replicated four times. A single *R. venosa* was randomly allocated to a circular experimental aquarium for 24 h prior to starting an experiment, after which 18 clams (or 18 mussels or 18 oysters) were placed in the aquarium. Each trial lasted 6 days, and the behavior of *R. venosa* was recorded for the first 96 h. Two aquaria without predators were used as controls to monitor natural prey mortality (no deaths occurred over 6 days).

Collection and statistical analysis of data

Predation and selectivity data

In all experiments, each aquarium was surveyed twice daily (at 08:00 a.m. and 20:00 p.m.) to count the number of prey eaten by the predator. The consumed prey was replaced with a live individual of the same species. In the single-prey experiments, the predation rate for each replicate was calculated as the number of prey animals eaten per day per predator. In the two-prey and three-prey experiments, the total numbers of each prey species eaten by the predator were counted to calculate the selection index, as follows:

$$\alpha_i = \frac{r_i/n_i}{\sum_{j=1}^q r_j/n_j} \times 100\%, \quad i = 1, \dots, q \quad (\text{Chesson 1978})$$

where α_i is the selection index for prey type i , r is the number of prey eaten by the predator, n is the total number of prey in the aquarium, and q is the total number of prey types in the environment (Chesson 1978).

The number of each prey type eaten in the two-prey experiment (choice situation) was designated as the observed frequency; the number of prey eaten in the single-prey experiment (non-choice situation) was designated as the expected frequency. If prey selection was observed, active selection was examined by comparing observed frequencies to expected frequencies using the Chi-square test with Yates's correction for continuity or Fisher's exact test (Liszka and Underwood 1990; Zar 1996). Expected frequencies were calculated as:

$$E_i = R \left(\frac{S_i}{S_i + S_m} \right)$$

$$E_m = R \left(\frac{S_m}{S_i + S_m} \right)$$

where E_i and E_m are the expected numbers of prey type i and prey type m eaten, respectively, in predator i - m combination; R is the number of prey type i plus the number of prey type m eaten when presented together; and S_i and S_m are the numbers of prey type i and prey type m eaten when presented alone, respectively (Liszka and Underwood 1990). Active selection was evident when the Chi-square value was significant.

Collection of behavioral data

Behavioral data for *R. venosa* during the first 96 h were acquired using a video recorder (model DS-2CD864-EI3, China) fixed 1 m above the water surface and stored in the recorder (Hikvision, DS-7604 N, China) for further analysis. Predator behaviors were quantified during continuous 96-h periods for each treatment. Predator foraging behaviors included searching for and handling the prey (Barbeau and Scheibling 1994; Wong and Barbeau 2003; Sun et al. 2015). The proportions of searching and handling time were calculated as: total searching time/total observation time, and total handling time/total observation time. In this study, searching time was estimated as the total time from predator search beginning to encounter with a prey animal; the handling time was estimated as the total time taken by the predator to manipulate and consume a single prey animal, from encounter to consumption ending. We also quantified the individual prey-handling times in each treatment.

For *R. venosa*, there were four outcomes that were commonly observed after an encounter: avoidance, pre-capture rejection, rejection (post-capture) and consumption. Encounters between predator and prey, and the subsequent outcomes of the encounters, were quantified (Wong and

Barbeau 2003). In this study, encounter rate was calculated as the total number of encounters/total searching time (h^{-1}). The probability of attack upon encounter, $\text{Pr}\{\text{attack} \mid \text{encounter}\}$, was calculated as [(encounters - avoidances)/encounters]; the probability of capture upon attack, $\text{Pr}\{\text{capture} \mid \text{attack}\}$, was calculated as [(rejections + consumptions)/(pre-capture rejections + rejections + consumptions)]; the probability of consumption upon capture, $\text{Pr}\{\text{consumption} \mid \text{capture}\}$, was calculated as [consumptions/(rejections + consumptions)]. Individual prey-handling times of *R. venosa* in each treatment were counted on the video recording. The foraging behavior of *R. venosa* is described in more detail in Results section.

Predator behavior sequences were examined using ethograms, specifically considering the states: (1) stationary, (2) moving and not foraging, (3) searching and (4) prey-handling (Nadeau et al. 2009; Sun et al. 2015). Relative frequencies of transition were calculated from the number of transitions between two behaviors divided by the total number of transitions. This analysis and the associated kinematic graphs were performed for each *R. venosa*-clam, *R. venosa*-mussel and *R. venosa*-oyster combination. The kinematic graphs were drawn using Microsoft Office Visio 2003 software to assist the behavior analysis.

Collection of prey characteristics data

To better understand the mechanisms underlying observed prey selection, prey characteristics (energy content, handling time per prey, prey profitability, shell strength) were measured (Wong and Barbeau 2005). To quantify energy content per prey, tissue samples from clams, mussels and oysters ($n = 30$) were dried for 48 h at 80 °C. Mean dry weight per prey item was calculated from the number of prey items divided by the total dry weight. Mean energy per unit dry weight was measured using an oxygen bomb calorimeter (Parr Instrument Company). The energy content per prey item was calculated as the mean dry tissue weight multiplied by the mean energy content per unit dry weight. Handling time per prey item was measured during video analysis. Prey profitability was calculated from its mean energy content divided by mean handling time per prey item (Stephens and Krebs 1986). A Shore hardness tester was used to measure the shell strength of the three prey species.

Statistical analysis

The selectivity indices were analyzed using an independent sample T test. Predation rates, behavioral datum (proportions of searching time, proportions of handling time, encounter rates, probabilities of attack upon encounter,

Table 3 Energy content, handling time per prey, profitability and shell strength of three prey species

Prey species	Energy content (kJ prey ⁻¹)	Handling time per prey (h prey ⁻¹)	Profitability (kJ h ⁻¹ prey ⁻¹)	Shell strength (HD)
Clam	7.08 ± 0.12 ^c	2.90 ± 0.53	2.49 ± 0.42 ^b	72.53 ± 1.19 ^b
Mussel	3.53 ± 0.11 ^b	6.26 ± 2.52	0.68 ± 0.36 ^a	41.85 ± 2.70 ^a
Oyster	2.04 ± 0.34 ^a	6.53 ± 2.23	0.34 ± 0.16 ^a	83.57 ± 1.35 ^c

Values without the same superscript in the same row were significantly different from each other ($P < 0.05$)

probabilities of capture upon attack and probabilities of consumption upon capture), transitions between the four major behaviors, searching events (stationary to searching, moving to searching and handling to searching) and prey characteristics (energy content, handling time per prey, prey profitability and shell strength) were analyzed using one-way ANOVA. Levene's test was used to test the assumption of homogeneity of variances. Square root transformation of the probability of attack upon capture was performed to obtain homogeneity of variance. For multiple comparisons, Duncan's test was used to compare the means. Probabilities of transition in the ethograms for predator–clam, predator–mussel and predator–oyster combinations were compared using the independence test (Chi-square test). All data are reported as mean values ± standard deviation (mean ± SD). Statistical analyses were performed using SPSS 22.0 software.

Results

Prey characteristics

The energy contents, handling times per prey item, profitability and shell strengths of clams, mussels and oysters are shown in Table 3. There were significant differences among the three prey species in energy content ($MS_t = 20.83$, $MS_e = 0.046$, $F_{2,6} = 438.079$, $P < 0.001$). The mean energy content per clam was significantly higher than per mussel, and the mean energy content per mussel was significantly higher than per oyster. There were no significant differences among the handling times of clams, mussels and oysters ($MS_t = 12.717$, $MS_e = 4.218$, $F_{2,7} = 3.015$, $P = 0.114$). For profitability, there were significant differences among the three prey species ($MS_t = 4.121$, $MS_e = 0.114$, $F_{2,7} = 36.289$, $P < 0.001$). Profitability was significantly higher for clams than for mussels and oysters, but there was no significant difference between mussels and oysters. There were significant differences among the three prey species in shell strength ($MS_t = 1869.62$, $MS_e = 14.08$, $F = 132.787$, $P < 0.001$). It was significantly lower for mussels than for clams and oysters, and the shell strength of oysters was significantly higher than that of clams.

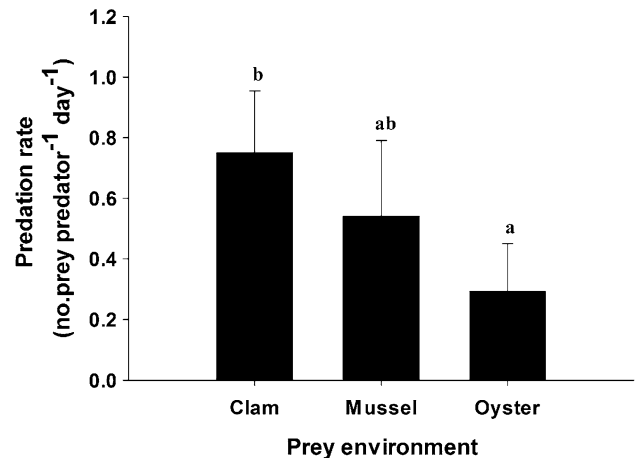


Fig. 1 Predation rate of *R. venosa* prey on clams, mussels and oysters

Predation rates and prey selection

In the single-prey experiments, the predation rates of *R. venosa* on clams, mussels and oysters were 0.75 ± 0.21 , 0.54 ± 0.25 and 0.29 ± 0.25 day⁻¹, respectively (Fig. 1). There was a statistically significant difference among clams, mussels and oysters consumed by *R. venosa* ($MS_t = 0.210$, $MS_e = 0.043$, $F_{2,9} = 4.857$, $P = 0.037$). The predation rate on clams was significantly higher than on oysters, but the differences between clams and mussels, and between mussels and oysters, were not significant. In the three-prey experiment, no oysters were consumed by *R. venosa* and the predation rates on clams and mussels were 1.25 ± 0.35 and 0.13 ± 0.14 day⁻¹.

The prey selectivity indices in the RCO combination are shown in Fig. 2a. The clam selectivity index was 1, and the oyster selectivity index was 0; that is, only clams were consumed by *R. venosa* in this combination. In the RCM combination (Fig. 2b), the clam selectivity index (0.83 ± 0.12) was significantly higher than the mussel selectivity index (0.17 ± 0.12) ($t_6 = 7.979$, $P < 0.001$). In the RMO combination (Fig. 2c), when mussels and oysters were presented together, only mussels were consumed; that is, in this combination, the mussel selectivity index was 1 and the oyster selectivity index was 0. In the RCMO combinations (Fig. 2d), the oyster selectivity index was 0 and

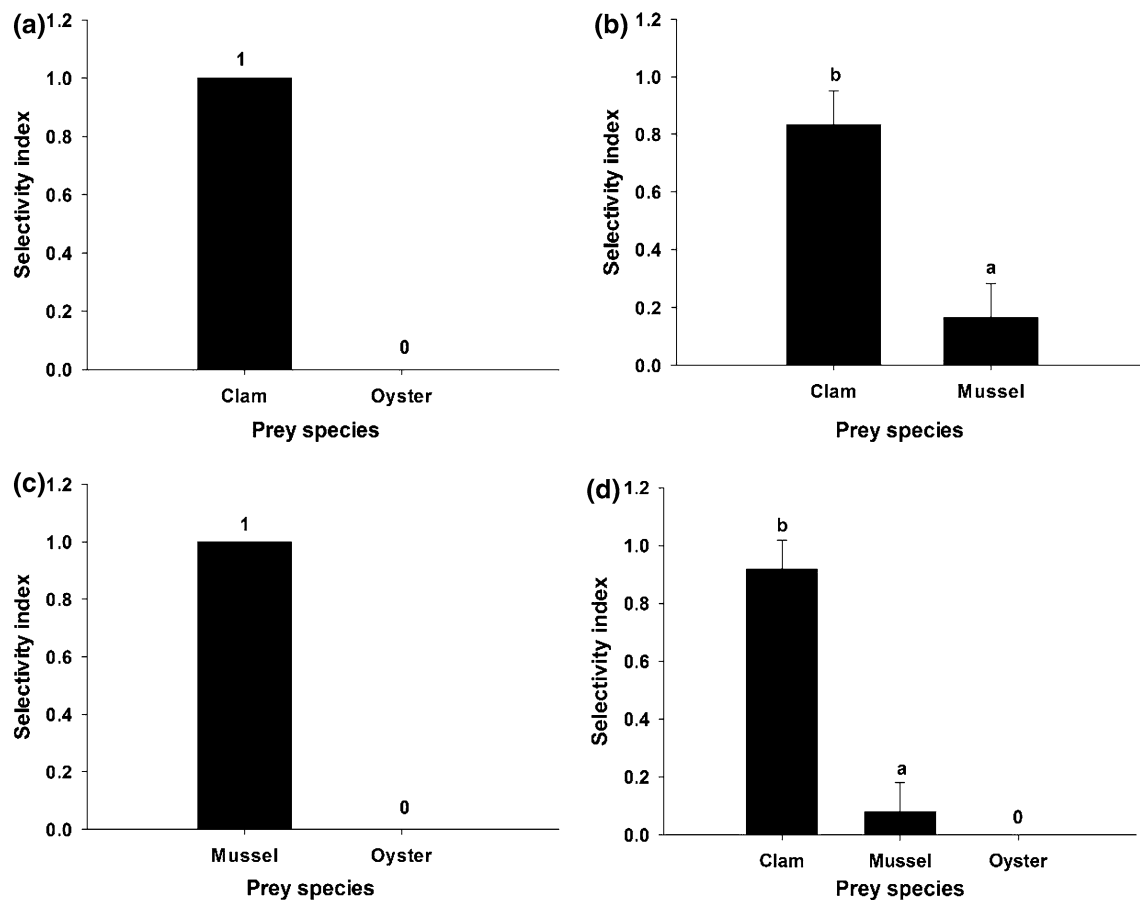


Fig. 2 Prey selectivity index in RCO, RCM, RMO and RCMO combination

Table 4 Results of Chi-square test comparing numbers of preys consumed in two prey type experiments (observed frequencies) to the expected frequencies calculated from numbers of preys consumed in the single-prey type experiments

Combination	Prey type	Observed frequencies	Expected frequencies	Value	<i>P</i>
RCO	Clam	25	15.8	8.672	0.003 ^a
	Oyster	0	9.2		
RCM	Clam	17	10	5.584	0.018
	Mussel	3	10		
RMO	Mussel	13	8.2	6.190	0.039 ^b
	Oyster	0	4.8		

Expected frequencies were calculated using the number of prey consumed during the first 4 days in no choice system

^a The probability of Yate's correction for continuity

^b The probability of Fisher's exact test

the clam selectivity index (0.92 ± 0.10) was significantly higher than the mussel selectivity index (0.08 ± 0.10) ($t_6 = 12.001$, $P < 0.001$). *R. venosa* strongly selected clams over mussels and oysters in all experiments.

Active selection was detected underlying observed selection, and the results are shown in Table 4. The numbers of clams, mussels and oysters consumed in the two-prey

experiments differed significantly from the expected numbers calculated from the single-prey experiments, indicating that *R. venosa* actively selected one type of prey when given a choice. When clams were presented together with oysters or mussels, *R. venosa* actively selected clams. In the presence of oysters and mussels, *R. venosa* actively selected mussels.

Foraging behavior

Behavioral observations

When not foraging, *R. venosa* remained quiescent on the bottom of the aquarium or on the wall near the water surface, or moved in a circular route. The foraging behavior was similar for the three bivalves used in this study. *R. venosa* searched for prey by probing the bottom and moving slowly with the foot. The search path changed with the position of the prey. Actual contact between *R. venosa* and a prey animal was considered an encounter. Upon encountering a prey, *R. venosa* attacked with the foot flipping over the prey or pushing the shell of the prey. Escape behavior was not observed by any of the prey species in this study. Avoidance by *R. venosa* was considered to have occurred when its foot or shell lightly touched the edge of the shell of an encountered prey, and it moved away. Capture was deemed to have occurred when the prey was covered by the foot of the whelk. Handling of the prey began when the whelk completely enclosed the prey and ended when the whelk rejected or consumed the prey. In this study, the whelk consumed prey by boring into the edges of the shell valves or by smothering the prey, and then digesting its tissues.

Encounter rate between *R. venosa* and prey

Encounter rates with clams, mussels and oysters are shown in Fig. 3 ($MS_t = 50.927$, $MS_e = 6.610$, $F_{2,9} = 7.705$, $P = 0.011$). The values were 5.08 ± 1.87 , 8.91 ± 3.38 and 12.2 ± 2.22 prey items per search time for clams, mussels and oysters, respectively. The encounter rate with oysters was significantly higher than with clams; there were no significant differences between mussels and clams, or between

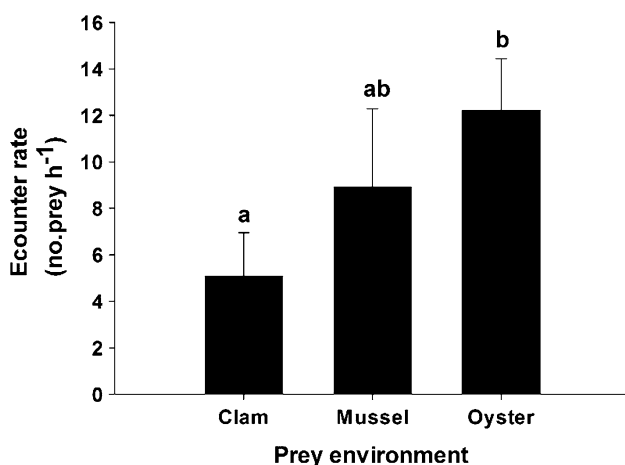


Fig. 3 Encounter rate of *R. venosa* with clams, mussels and oysters

mussels and oysters. *R. venosa*'s search efficiency was higher when offered oysters than offered clams.

Encounter behaviors between *R. venosa* and prey animals

In the single-prey experiments, the probabilities of attack upon encounter of *R. venosa* with clams, mussels or oysters were 72 ± 33 , 62 ± 15 and 50 ± 12 %, respectively (Fig. 4a), and there were no significant differences among these groups ($MS_t = 0.017$, $MS_e = 0.020$, $F_{2,9} = 0.811$, $P = 0.475$). This behavior component appeared not to be an important component of the observed selection.

The probabilities of capture upon attack by *R. venosa* on clams, mussels or oysters were 100, 84 ± 21 and 46 ± 18 %, respectively (Fig. 4b) ($MS_t = 0.306$, $MS_e = 0.025$, $F_{2,9} = 12.401$, $P = 0.003$). All attacked clams were captured by *R. venosa*, but about half attacked oysters were rejected. The probabilities of capture upon attack for clams and mussels were significantly higher than for oysters. The probability of capture upon attack for clams was not significantly different than for mussels. This behavior component was the main mechanisms determining observed selection of *R. venosa* for three prey species.

The probabilities of consumption upon capture of clams, mussels or oysters were 80 ± 14 , 35 ± 16 and 23 ± 5 %, respectively (Fig. 4c) ($MS_t = 0.0362$, $MS_e = 0.016$, $F_{2,9} = 22.286$, $P < 0.001$). *R. venosa* rejected captured oysters relatively often (1–23 % of captures), but consumed almost all captured clams. The probability of consumption upon capture was significantly higher for clams than for mussels and oysters. There was no significant difference between the mussels and the oysters. This behavior component was the main mechanisms determining observed selection of *R. venosa* for three prey species.

R. venosa foraging time budget

Rapana venosa spent 1.23 ± 0.52 , 2.28 ± 1.23 and 3.08 ± 0.35 % of their time searching for clams, mussels and oysters, respectively (Fig. 5a) ($MS_t = 3.5 \times 10^{-4}$, $MS_e = 0.6 \times 10^{-4}$, $F_{2,9} = 5.461$, $P = 0.028$). The time budget of searching for oysters was significantly greater than for clams; there were no significant differences between mussels and clams, or between mussels and oysters.

The relative handling times for the different prey are shown in Fig. 5b ($MS_t = 0.073$, $MS_e = 0.014$, $F_{2,9} = 5.130$, $P = 0.033$). *R. venosa* spent 35.97 ± 15.23 % of their time budget handling mussels, which was significantly higher than handling clams (about 9.05 ± 3.25 %). They spent 20.52 ± 13.56 % of their time budget handling oysters, which was not significantly different from clams or oysters. Handling time budgets were inconsistent among the three

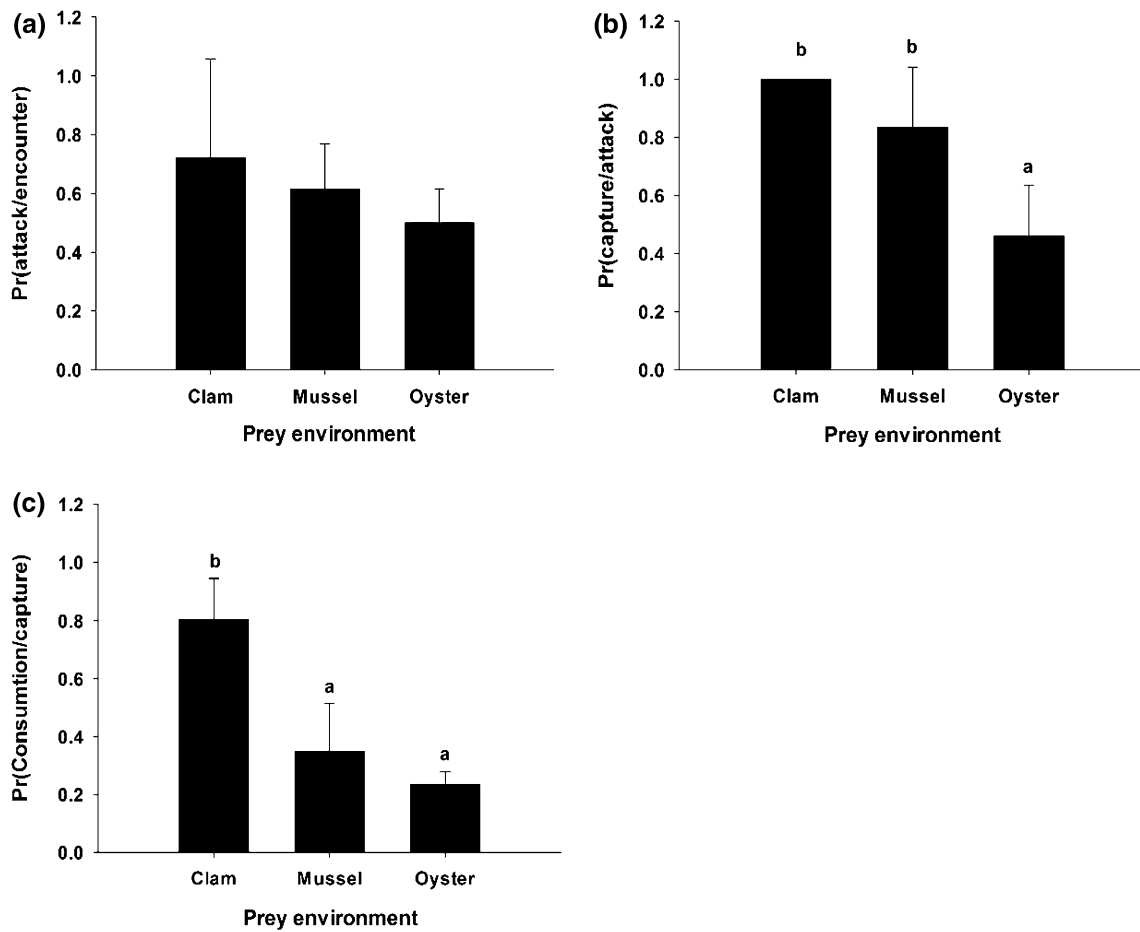


Fig. 4 Probability of attack upon encounter (a), the probability of capture upon attack (b) and the probability of consumption upon capture (c) between *R. venosa* and preys

prey species. This variability may be related to the different

methods used to open the bivalve prey by *R. venosa*.

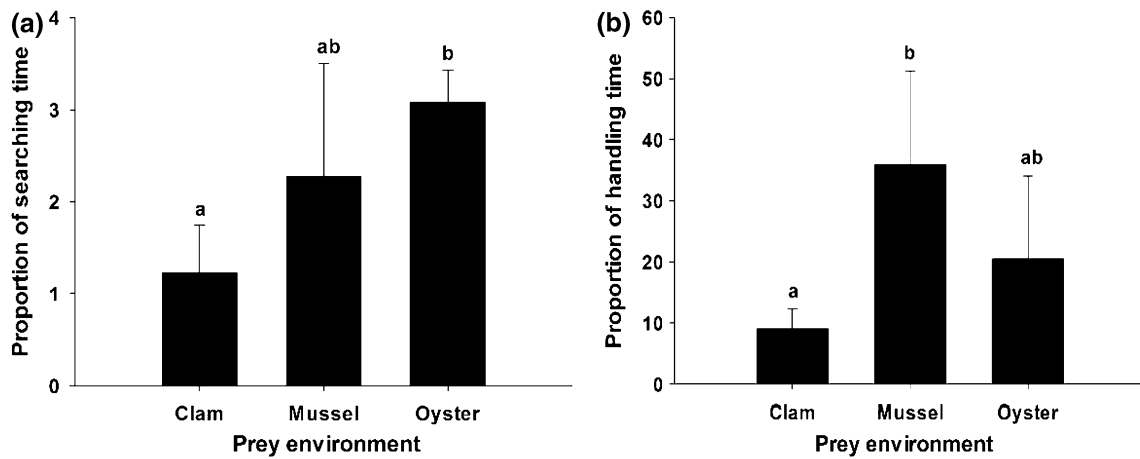


Fig. 5 Mean proportion of time that *R. venosa* spent searching (a) and handling (b) for clams, mussels and oysters

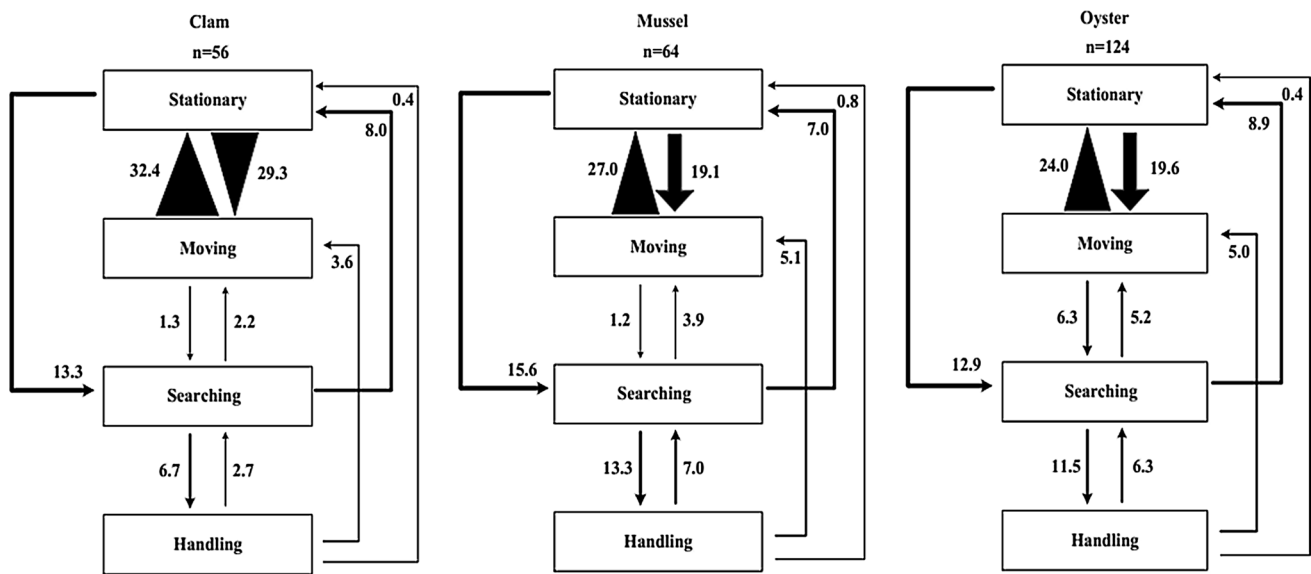


Fig. 6 Kinematic graphs of the behavioral sequences of *R. venosa* offered clams (*R. philippinarum*), mussels (*M. edulis*) and oysters (*C. gigas*). The thickness of arrows and the value beside indicate the relative frequency between two behaviors; n represents total number of transitions

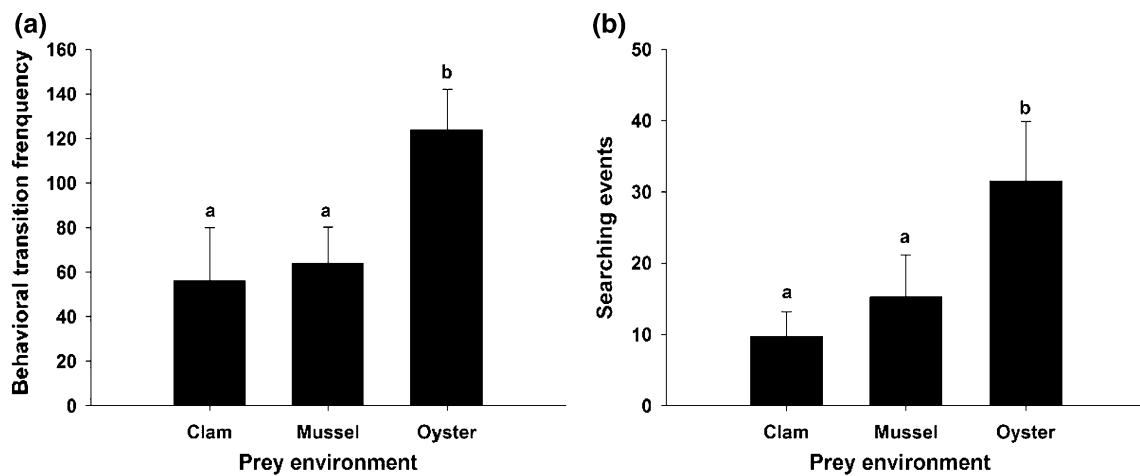


Fig. 7 Transitions between all four major behaviors (a) and searching events (b) of *R. venosa* in the *R. venosa*–clam, *R. venosa*–mussel and *R. venosa*–oyster system

Ethograms of R. venosa foraging clams, mussels and oysters

Ethograms for *R. venosa* preying on clams, mussels and oysters are shown in Fig. 6. The main transitions in the ethograms were between stationary state, moving and searching. The most frequent transitions were from moving to being stationary (24–32.4 %) and from being stationary to moving (19.1–29.3 %). The third and fourth most frequent change were from being stationary to searching (12.9–15.6 %) and searching to being stationary (7.0–8.9 %). In addition, handling behavior only transitioned

from searching behavior. Predation behavior was linear and rarely by-passed the main sequence. Transitions between the four major behaviors in the *R. venosa*–oyster system (124 ± 18.0) were significantly higher than in the *R. venosa*–clam system (56 ± 23.8) and *R. venosa*–mussel system (64 ± 16.2) ($MS_t = 5500.08$, $MS_e = 385.19$, $F_{2,9} = 14.279$, $P = 0.002$) (Fig. 7a). There were no significant differences among the three kinematic graphs (Table 5), although there were significantly more searching events (stationary to searching, moving to searching, handling to searching) for oysters (31.5 ± 8.3) than for clams (9.8 ± 3.4) or mussels (15.3 ± 5.9) ($MS_t = 511.58$,

Table 5 Independence test (Chi-square test) of ethograms

Combination	<i>df</i>	Value	<i>P</i>
RC versus RO	6	7.197	0.303
RM versus RO	6	3.114	0.794
RC versus RM	6	4.057	0.669

Only seven transitions were used in this test (stationary–moving; moving–stationary; stationary–searching; moving–searching; searching–stationary; searching–moving; searching–handling)

$MS_e = 38.50$, $F_{2,9} = 13.288$, $P = 0.002$) (Fig. 7b). It suggests that *R. venosa* would be more active when preyed on oysters than on clams and mussels.

Discussion

Prey selection and foraging behavior

Rapana venosa strongly selected clams (*R. philippinarum*) when all three prey species were offered concurrently. *R. venosa* actively selected particular prey species when offered two species of prey concurrently. Clams were always selected when presented together with mussels (*M. edulis*) or oysters (*C. gigas*), and *R. venosa* selected mussels when offered mussels and oysters concurrently. In single-prey experiments, clams were generally consumed in the greatest number by *R. venosa*. These results imply that *R. venosa* preferred clams, followed by mussels and oysters. We predicted that *R. venosa* would select clams over mussels and oysters and our predictions were supported. In our study, handling time per prey did not provide a useful framework to understand selection since it did not differ much among clams, mussels and oysters; differences in energy content resulted differences in profitability (Table 3). From energy analyses, prey selection of *R. venosa* may be based on selection of prey with the highest energy content or profitability. The selection of clams is consistent with the optimal foraging theory (Hughes 1980) that predators actively select prey to maximize the rate of energy intake. In addition, proportion of time spent searching for clams was lower than mussels and oysters. These results confirm previous study made by Savini and Occhipinti-Ambrogi (2006), who tested prey preference of *R. venosa* in the Northern Adriatic Sea. They found that *R. venosa* behaved as a “pursuer” searched and consumed for specific prey (*S. inaequalvis*), thus maximizing its net rate of energy intake.

Active selection was detected since *R. venosa* always consumed significantly more clams than expected when given a choice of prey types than when not given a choice

(Table 4), suggesting that predation events (encounter, attack, capture and consumption) may associated with active selection by the *R. venosa*. In our study, the probability of attack upon encounter did not vary among prey types and so did not help to explain predation patterns. Despite the lowest encounter rate between whelks and clams, predation rates on clams were the highest. Therefore, encounter rates between whelks and prey types were unlikely to be important in such small mesocosms as those we used. Prey selection by *R. venosa* appeared to be due to a combination of the probability of capture and the probability of consumption. Both the probability of capture upon attack and the probability of consumption upon capture were the highest for clams (Fig. 4). In other words, clams experienced the highest predation rates because they were easier to capture and consume than mussels and oysters. According to the literature data, juvenile and small-sized *R. venosa* are feeding by drilling through the bivalve shell, whereas large snails can attack and consume bivalves use two methods edge drilling and suffocation (Chukhchin 1984; Harding et al. 2007; Kosyan 2015). Rapa whelks were large enough to manipulate their prey with the latter two methods in our study. Clams with smooth and low strength shell were easy to be wrapped (capture) by the foot of whelks and to be opened by edge drilling (consume). Oysters have the highest shell strength; therefore, they are difficult to drill by *R. venosa*. In addition, difficulty also comes from that the shell of oysters always tightly closed (Dietl 2003). The lowest probability of capture and consumption and highest handling time for oysters mostly likely resulted from the anti-predatory behavior and characteristic of oysters. In other words, oysters are less vulnerable and accessible to the predation by *R. venosa* than clams and mussels.

The encounter rate between *R. venosa* and clams was significantly lower than for oysters although the predation rate on clams was significantly higher than on oysters (Figs. 1, 3). This result may be explained by satiation. Whelks offered clams quickly reached satiation, whereas those offered oysters did so more gradually. Hughes and Dunkin (1984) found that hungrier dogwhelks *Nucella lapillus* crawled faster in straighter paths and spent more time for searching and less hunger dogwhelks would tend to restrict their searching close to original site. In our study, whelks offered oysters spent more time searching for prey (Fig. 2a) and improved search efficiency (encounter rate) to regulate starvation. This finding suggests that *R. venosa* applies similar searching strategy with dogwhelks to regulate starvation. Based on our comparison of the ethograms of *R. venosa* in different prey environments, transitions between the four major behaviors in the *R. venosa*–oyster system were much more frequent than in the *R. venosa*–clam system and *R. venosa*–oyster system (Figs. 6, 7a).

Searching events in the *R. venosa*–oyster system were also significantly more frequent than in the *R. venosa*–clam system and *R. venosa*–mussel system (Fig. 7b). These results indicated that foraging activities were reinforced to regulate starvation when offered oysters. We speculate that *R. venosa* would more active when preying on non-preferential prey than preferential prey.

Until recently, sequential behavioral analysis using ethograms has rarely been used with marine animals (but see Himmelman et al. 2005; Nadeau et al. 2009; Sun et al. 2015). This behavioral approach provides a synoptic overview of the major behavioral transitions that occur within a predator–prey system and could complement or support analyses resulting from simple behavioral observations (Nadeau et al. 2009). The predation patterns of *R. venosa* on three prey species were similar (Table 4). Transitions mainly took place between stationary, non-foraging displacement and searching. *R. venosa* predation behavior was linear and rarely by-passed the main sequence. This predation pattern was similar to that of sea stars studied by Nadeau et al. (2009). Rapa whelks and sea stars both move slowly ($<10 \text{ cm min}^{-1}$) and consequently have low searching efficiency and handle each prey animal slowly (3 h~), which may explain the similarity.

Implications for practice and benthic communities

Our study has applications for enhancement of commercial bivalves. In single-prey experiments, all three prey species were consumed, substantially confirming *R. venosa*'s broad dietary capabilities (Mann and Harding 2003). Our study suggests that if *R. venosa* is the main predator of seeded oysters, additional mussels could be seeded for protect oyster, since mussels have a lower economic interest than clams. Mussels have a higher profitability and lower shell strength than oysters, which could lead to *R. venosa* active select mussels. In addition, clumps of mussels should be separated, because of interactions with clusters of conspecifics, which inhibit movement of the predators. However, increased prey density may result in aggregations of predators (Taylor 1984). Also, a new prey species may draw into new predator species such as crabs and sea stars. These potential issues must be taken care. In our study, all three prey species were offered with no refuge (sediment) to *R. venosa*. Actually, whether there is a refuge may influence the selection of *R. venosa*. Munari and Mistri (2011) studied the effect of short-term hypoxia on *R. venosa* prey preference. They offered three prey species: ark shell *Scapharca inaequivalvis* and Manila clam *Tapes philippinarum* and cockle *Cerastoderma glaucum*, and found that *R. venosa* preferred *S. inaequivalvis* under normoxia, while preferred *T. philippinarum* after short-term hypoxia, since clams migrated vertically into the sediment, to regulate

hypoxia, becoming more vulnerable to the predator. So, further study with the sediment or specific study in the filed should be implemented.

The interaction between predator and prey likely influences the abundance and distribution of predator and prey species. It is interesting that natural populations of mussels are almost absent from Laizhou Bay (can only be found on coastal rocky bottom). This may be attributed to the predation pressure from *R. venosa* due to a preference for this bivalve species. Conversely, no preference for oysters by *R. venosa* might explain the large numbers of this prey species. Sun et al. (2016) showed that natural Manila clams not only escape from predation by burrowing into sand but also hiding behind rock. Obviously, the latter way that clams use to avoid whelk predation has failed, since clams cannot be found in the rocky bottom community. This interaction might explain the distribution of clams that this species only abundant on the sandy bottom in the Laizhou Bay. Classical ecological theory suggests that prey share common enemies are unstable, leading to one species being eliminated from the interaction (Munari and Mistri 2011). The reason is often due to the different susceptibility of prey species to enemy, which results in one species “winning” and the other “losing” in the indirect interaction (Bonsall and Hassell 1997). The winning of the oyster in our study is determined by its higher shell strength and anti-predatory behavior. These prey characteristics make the oyster distribute on almost all rocky bottom in the Laizhou Bay. As the most abundant benthic predator, the large numbers of *R. venosa* may due to this species are probably capable of attacking virtually all components of the Bohai Sea invertebrate fauna.

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Compliance with ethical standards

Conflict of interest Authors declare that he/she has no conflict of interest.

Human and animal rights All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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