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Field and laboratory observation of Mediterranean mussel *Mytilus galloprovincialis* predation by flatworm *Stylochus mediterraneus*

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A R T I C L E I N F O

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A B S T R A C T

Recently, large numbers of Polyclad flatworms (*Stylochus mediterraneus*) have been observed in Istrian County shellfish farms connected to intensive ascidian fouling. To assess a possible threat and determine whether the flatworm feeds only on weakened dead mussels or can also infest healthy mussels, we have conducted a field research and laboratory mussel predation experiments. Performed field observations (August 2022–May 2023) indicated increased mussel mortality and, at the same time, increased abundance of flatworms from < 2 to 6/40 kg mussel ropes. Under laboratory conditions, flatworm feeding experiments with mussels (*Mytilus galloprovincialis*) and invasive tunicate species (*Clavelina oblonga*), showed a clear preference of flatworms for mussels. Using a newly developed valve gaping monitoring system (VGMM), based on an Arduino microcontroller platform and Hall sensors, we monitored valve gaping (VG) of mussels during acclimatisation (one day) and after exposing them to flatworms until the death of the first mussel (two days). Before and during exposure of mussels to flatworms, we were able to observe the normal daily rhythm of mussels VG (> 70 % time filtrating with the valve open > 50 %, with 1–5 resting periods), the behavioural changes of exposed mussels compared to control mussels, the timing of flatworm attack, the response of mussels during predation, and the timing of mussel death. We conclude that the flatworm *S. mediterraneus* is most likely a very opportunistic species that prefers weaker mussels but can also feed on healthy mussels. This investigation brings new knowledge and assumptions on the behavioural patterns of investigated flatworms. Further studies should consider that "experienced" flatworms attack and kill the mussels, while the rest simply eat a free meal using the strategy of the predator pack.

1. Introduction

Mussel *Mytilus galloprovincialis* is an important commercial mariculture species and strong bioindicator commonly used to monitor environmental conditions, spatial distributions and temporal trends of pollutants in coastal and estuarine areas (Gosling, 1992; Hamer et al., 2008; Kanduč et al., 2011). Following the intensive occurrence of the invasive ascidian tunicate *Clavelina oblonga* Herdman, 1880 fouling in 2021 and 2022 at shellfish farm locations in Lim Bay (NE Adriatic Sea, Croatia) described by Majnarić et al. (2022), numerous individuals of the free-living (turbellarian) flatworm *S. mediterraneus* Galleni (1976) appeared in summer-autumn 2022. Polyclad flatworms, commonly called wafers or oyster leeches, are a pest to shellfish culture globally. It was previously established that *S. mediterraneus* feeds on commercial

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mussels and oysters (Gammoudi et al., 2017). Different approaches were made to develop an efficient method for their control, including experimentation by prey selection depending on flatworm size (O'Connor and Newman, 2002; Gammoudi et al., 2017).

More recently, behavioural markers have been applied to assess changes in the health status of mussels and the ecosystem in response to different threats and as a part of biological early warning systems (BEWSs), e.g. Mosselmonitor® (de Zwart et al., 1995) and Dreissena--Monitor® (Borcherding, 2006). The mussel valve movement (gaping) is widely recognised as an integrative measure of physiological functions such as respiration, feeding and excretion (Gnyubkin, 2010; Przymus et al., 2014), which can change under stressful conditions in response to a deteriorating environment and as a defence reaction to external stimuli, e.g. touching, shading, or the sudden approach of a predator (Cameau et al., 2018). For example, toxic red tides, oxygen deficiency, low salinity, or elevated water temperatures have been shown to induce an abnormal valve gaping (Gainey and Shumway, 1988; Baldwin and Kramer, 1994; de Zwart et al., 1995; Rajagopal et al., 1997; Kramer and Foekema, 2000; Kramer, 2009; Dowd and Somero, 2013). Therefore, quantifying valve movements (i.e. recurrence of opening and closure of shell) and gaping (i.e. the distance between two valves of the shell) under a variety of natural and experimental conditions can aid in understanding the general physiological responses of these organisms to abiotic stresses in the environment (Burnett et al., 2013; Beggel and Geist, 2015; Lummer et al., 2016), biotic interactions (Rovero et al., 1999; 2000), and exposure to toxins (Kiyohito et al., 2006; Redmond et al., 2017).

Conventional methods of measuring valve movements include kymographic and strain-gauge methods (Higgins, 1980), electromyography (Jenner et al., 1989), impedance electrodes (Tran et al., 2004), laser sensors (Redmond et al., 2017), and magneto-electric devices (Kramer and Foekema, 2000; Wilson et al., 2005; Robson et al., 2007). Magneto-electric device instruments assess the valve movements in the form of the output voltage from the Hall element sensor on one shell, generated by changes in the external magnetic field from a magnet attached to the other. Such technology with the Hall sensor system has been used to study valve gape behaviour of the mussel *Mytilus trossulus* and *M. edulis*, the scallop *Pecten maximus*, and the cockle *Cerastoderma edule* (Robson et al., 2010; Clements and Comeau, 2019) especially after the affordable microcontroller Arduino platform with dozen available sensors appeared on the market. Magneto-electric systems have been applied in the Mediterranean mussel *M. galloprovincialis* Lamarck, 1819 for studying the effect of circadian rhythms on valve movements (Gnyubkin, 2010), valve-gaping behaviour of raft-cultivated mussels in the Ria de Arousa, Spain (Cameau et al., 2018), and flash flood simulation event of drastic and sudden reduction of seawater salinity decrease (Addis et al., 2020).

Until now, there is no data on *Mytilus* spp. VG response - behaviour after exposure to flatworm predation (Lupo et al., 2020). Flatworms have complex chemical defence and attack armour (toxins) able to paralyse their prey (McNab et al., 2021). However, besides the description of a hypothetical flatworm attack, entering the mussel shell cavity by detaching the adductor muscle of the mussel, the mechanism of flatworm entering into the mussel is still under speculation (Gammoudi et al., 2017). In addition to the field observations of mussel growth, vitality and mortality, we assessed the intensity of overgrowth (*C. oblonga*) and associated it with the presence of flatworms as a possible cause of mussel mortality. By exposing mussels and ascidian colony zooids to predation by flatworms in the laboratory, we also determined their food preference and performance. Finally, by applying VG mussel monitoring, we tried to answer the question: Do the flatworms *S. mediterraneus*, which hide in the fouling "sods" of *C. oblonga*, only eat weakened or dead mussels or do they also attack healthy mussels, and what strategy do they use?

2. Materials and methods

2.1. Field mussel growth and mortality observation

Monitoring of growth, vitality and mortality of mussel *Mytilus galloprovincialis* as well as observation of fouling by *C. oblonga* and predation of mussels by *S. mediterraneus* were conducted at the Navi mussel production site in Lim Bay from August 2022 to May 2023 (Fig. 1).

Field work began in August 2022 (Fig. 2) with the processing of commercial mussels threaten by *C. oblonga* overgrowth. We used only undersized mussels (< 6 cm) to produce culture ropes containing an average of 40 kg of mussels and 10 culture ropes in total. Further field observations were carried out in December (2022) and May (2023). After initial mussel vitality, mortality and overgrowth assessment (August 2022), *C. oblonga* was removed from the culture ropes/mussels and the regrowth of the newly established ascidian colonies was monitored accordingly (% overgrowth/culture rope) at new installations.

The growth and vitality of the mussels were evaluated by measuring the condition index - meat yield (3 × 10 representative specimens) and survival in the air test (SOS test, 30 mussels) according to Hamer et al. (2008). The abundance of flatworms was estimated by a detailed examination of the mussel ropes and overgrowth, whereby the number of specimens present was counted on three representative ropes (approx. 40 kg each). The mortality was determined by counting live specimens and empty/open shells. The average mussel mortality was expressed as a percentage compared to the total number of mussels in three representative culture ropes. The methods for growth, vitality and production performance of the mussels, including the number of samples, have been adapted to the needs of the shellfish farmers who monitor the mussels according to available resources and time.

2.2. Commercial mussel examination for possible infestation with polyclad flatworms

Following our field observations, we have conducted two detailed examinations of commercial mussels (3 biological repetitions, 5 kg of mussels/repetition, average mussel size 6–7 cm) from the Lim Bay shellfish farm Istrida d.o.o. (Figure 1, 2, 3, 5 and 6). Mussels prepared for sale at the local fish market were examined for infestation with the polyclad flatworm *S. mediterraneus* during June and August 2023. Each time, mussels (480 and 505 specimens) were individually examined for the presence of *S. mediterraneus* in the shells after the mussels were opened with a knife (in the field in June) or heated in the microwave (2 × 4 min) in the laboratory in August.

2.3. Field sampling and laboratory maintenance of target organisms

Specimens of the flatworm *Stylochus mediterraneus* Galleni (1976) and ascidian tunicate *Clavelina oblonga* Herdman, 1880 were collected at a mussel installation in the Lim Bay NE Adriatic Sea, Croatia (GPS 45° 07,992'N, 13° 41,937'E). The collected specimens were transferred in a bucket to the BioLab aquarium system (40 L tanks) with mechanical-biological filtration and UV-C treatment of the outflowing seawater. Before setting up the experiment, the species was confirmed using morphological keys described by Galleni (1976), and later taxonomically attributed according to Dittmann et al. (2019) (Fig. 3). The mussels *Mytilus galloprovincialis* Lamarck, 1829 were sampled from culture ropes at the same site and kept in a seawater flow-through system. Before transferring them to the BioLab aquarium system (40 L tanks) and exposure, we have cut off their byssus threads and cleaned the mussel shells. The BioLab experimental laboratory of the Center for Marine Research Rovinj (Ruđer Bošković Institute) is approved for experiments with marine fish (Croatian Ministry of Agriculture, HR-POK-029) and complies with the latest national regulations for experiments with marine fish and other organisms.

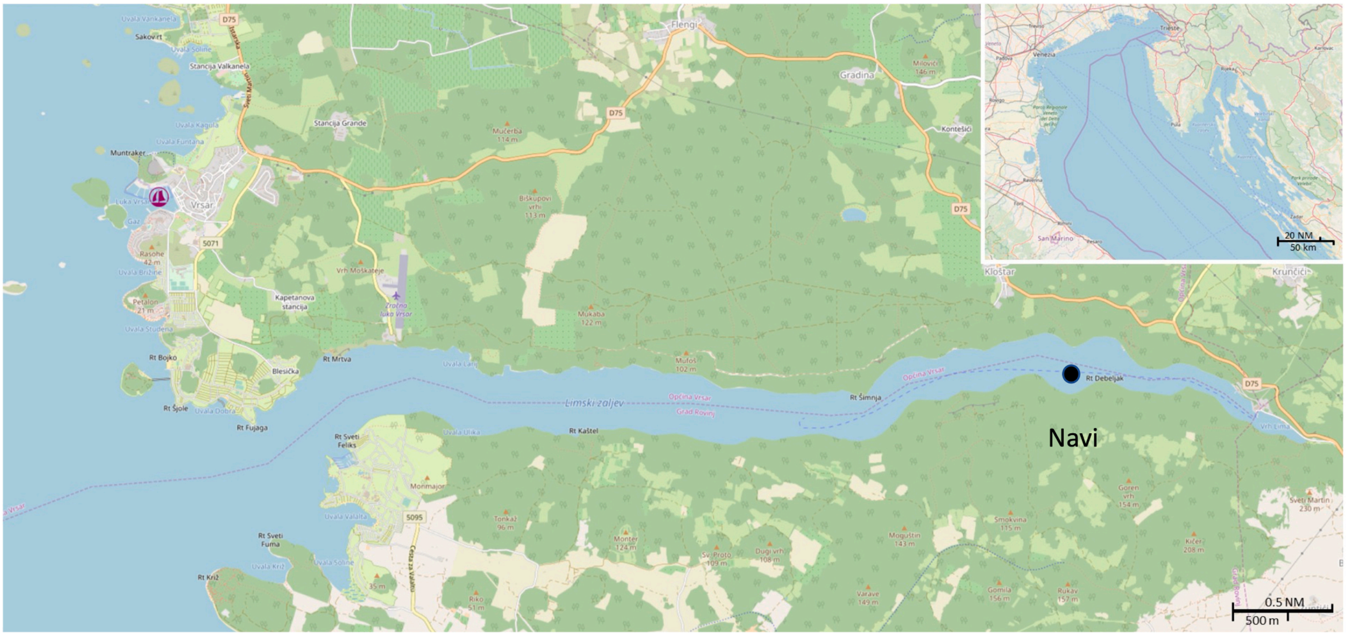

Fig. 1. Map of the study area in Lim Bay in the northeast Adriatic, Croatia, with the sampling and field observation site Navi (GPS 45º 07,992'N, 13º 41,937'E).

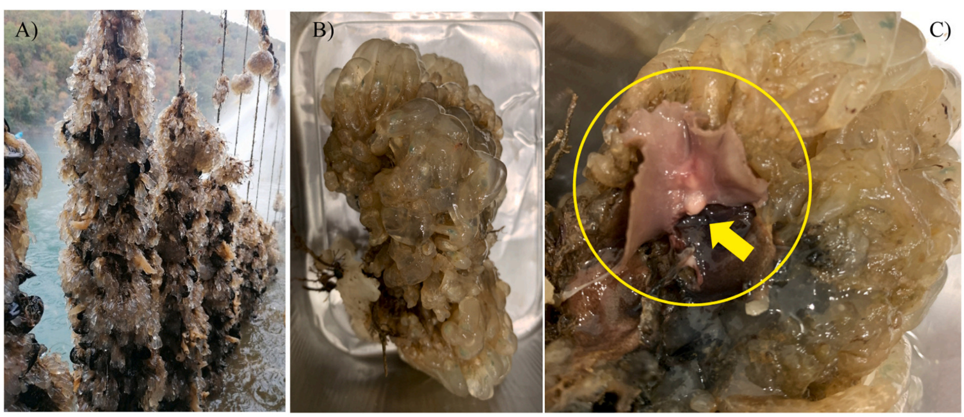

Fig. 2. Mussel production in Lim Bay mariculture threatened by the invasive alien species *Clavelina oblonga*: A) Shellfish farm infrastructure longline with mussel culture ropes; B) Ascidian colony body; C) Flatworm *Stylochus mediterraneus* (yellow circle) hiding in the ascidian nodule and probably feeding on the overgrowth (arrow).

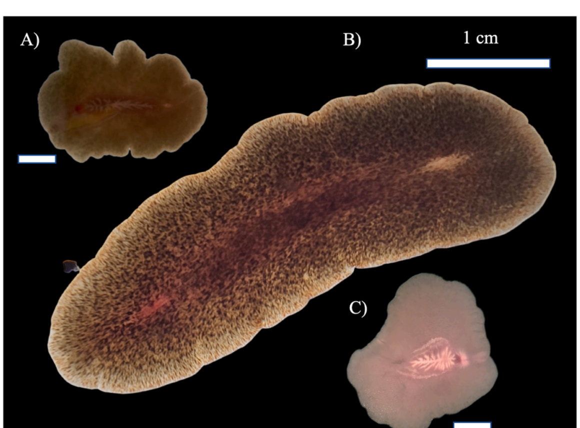

Fig. 3. The identification of the collected flatworm species as *S. mediterraneus* was based on the internal anatomy of the copulatory apparatus: A) and B) dorsal view, and C) ventral view. Each box corresponds to a size of 1 cm.

2.4. Laboratory determination of flatworm feeding preference and performance

Flatworm feeding preference experiments (*M. galloprovincialis* vs *C. oblonga*) were performed with 10 mussel specimens and 2 ascidian colonial corpora with 10 zooids each exposed to 10 specimens of flatworm (*S. mediterraneus*) in aquaria (40 L) for 4 weeks during two separate experiments in May and September 2023. Each day, the mussels and colonial corpora were checked for predation, open-eaten mussels were noted, while ascidian colonial zooids were weighted and observed for damage, providing additional data on flatworm feeding preference and performance.

2.5. Laboratory mussel predation experiment by mussel valve gaping monitoring

To observe the predation of mussels by flatworm *S. mediterraneus* and mussel valve gaping (VG) behavioural response, we used a developed Valve Gaping Mussel Monitor microcontroller system (VGMM) with six sensors (Fig. 4A) (Hamer, 2022). The developed VGMM measures the

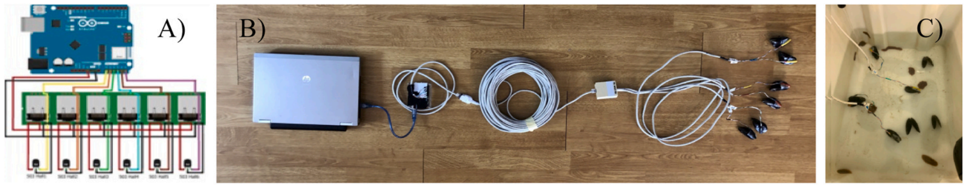

Fig. 4. Laboratory observation of mussel predation by flatworm *S. mediterraneus*: A) Arduino microcontroller and VG Hall sensors connection schema; B) VGMM with six sensors/mussels; C) Experimental setup (4 live mussels, 12 flatforms, 4 empty shells).

Fig. 5. Estimation of mussel mortality in Lim Bay mariculture - location Navi: A) Mussel in field culture rope/sock; B) Mussel mortality, collected empty shells after processing-cleaning of ropes; C) Flatworm *S. mediterraneus* hiding in mussel shell.

Fig. 6. Examination of live commercial mussels for infestation with flatworms: A) Mussels prepared for sale at the local fish market; B) External examination and C1) Internal visual inspection of raw (June) and C2) cooked (August) mussels.

valve gape (opening) using Hall sensors and magnet glued on opposite mussel shells close to the inhalant syphon. Opening and closing mussel shells and variation in gape extent produce a corresponding variation in the magnetic field strength measured by the Hall sensor. The intensity of the analogue Hall sensor signal was measured using an Arduino Uno microcontroller connected to a notebook and recorded by software (Fig. 4B) running in the shared Dropbox folder. Mussel VG activity was measured by a frequency of 0.5 Hz, generating 43,200 data records/day for one mussel (sensor). Normalisation of mussel VG data was necessary. Hall sensor output is proportional to magnetic field strength, but because of a small difference in sensor and magnet position, including mussel size (different valve gape), each sensor output was normalised by subtracting the minimum value to obtain zero for a closed mussel, and at the end of the experiment maximal VG value was used as 100 % opened mussel shell. For the presentation of mussel VG behaviour results, we chose a line graph of shell opening (0–100 %) during each day and a bar graph of cumulative daily mussel VG percent time occurrence (24 h, 9:00–9:00 h).

2.5.1. Experimental setup

After five days of mussel acclimation to experimental tanks conditions (temperature, 20 °C; salinity, 37; raw seawater exchange, 1 L/min; 12/12 h light and continuous aeration), Hall sensors and magnets were glued to opposite shells of each mussel using Aquarium silicon. In 2 × 40 L tanks, six (2 + 4) acclimated mussels (length 5.5 ± 0.1 cm; meat yield 19.4 ± 0.5 %) were connected to the VGMM. After drying (2 h), the equipped mussels connected to the microcontroller Arduino Uno and notebook were again placed in a flow-through aquarium system. Mussels were VG monitored one day to check normal valve gaping rhythm as a proxy for their filtration and resting activity. Afterwards, two mussels were used as control, and four mussels were exposed to 12 flatworm specimens and monitored for two days (Fig. 4C). Empty mussel shells (four empty shells) were added in the experimental tanks to imitate natural shelters of *S. mediterraneus*. Experiments with mussels and their VG response took place from 24th June to 15th July 2023. During the whole period of the experiment, the BioLab (temperature, lux) and basins (sea water temperature, salinity) were recorded using HOBO data loggers (U24-002-C and Mx2202) every 15 min, as well as daily seawater measurements using Hanna Instruments probe (HI98194).

2.6. Statistical analyses

The mussel condition index (meat yield) results are given as mean ±

S.D. of three biological repetitions each having 10 specimens. The mussel vitality (SOS test) was calculated by probit analysis and results are given as LT50 values with 95 % fiducial limits. The measured values of the mussel condition index and mortality between the different time samples were tested for significance level using the Welsch multiple comparation test (Welsch, 1977). Statistical significance was accepted at a $P < 0.05$ throughout (*). Mussel VG response after flatworm exposure was corelated to control values of typical mussel VG occurrence to check for behaviour deviation.

3. Results and discussion

3.1. Field mussel growth, vitality and mortality

The mussel production performance was estimated by determining mortality, counting live specimens and empty-open shells during the overgrowth/biofouling cleaning of mussel culture ropes during three observations (August 2022–May 2023) (Fig. 5). Mussel growth at the Lim Bay shellfish production site Navi had similar mussel meat yield during the observation period (August 2022–May 2023). All results of mussel condition indexes were within usual accepted meat yield values of 15–28 % for Lim Bay commercial mussels (Pavičić-Hamer et al., 2016). Higher initial overgrowth with *C. oblonga* decreased over time as a consequence of initial cleaning, while the LT50, mortality, and flatworm number increased (Table 1). More precisely, observations in December 2022 resulted with 20 % and in May 2023 with 30 % mortality with an average of 4.33 and 6 flatworm abundance per hanging nets (mussel culture ropes), respectively (Table 1). However, the increased numbers of open empty shells or dead mussels, were likely the result of higher predation and increased flatworm presence during the subsequent observation periods (Fig. 5). We can assume that initial intensive overgrowth competition (*C. oblonga* 80 % coverage) and high sea temperatures (27 °C) in August 2022 had a negative effect on mussel vitality (SOS test), but not on condition index (Table 1). According to Majnarić et al. (2022) higher populations of *C. oblonga* negatively affect and physically interfere with mussel growth and development. Another assumption is that removing of *C. oblonga* affected the occurrence of *S. mediterraneus* in mussel culture ropes by increasing their activity in predation and changing their patterns in requiring shelter and searching-attacking potential preys beside the mussels.

3.2. Commercial mussel examination for possible infestation with polyclad flatworms

Mussels prepared for sale on local fish market were checked for polyclad flatworm's infestation in June and August 2023 (Fig. 6). Each time approx. 500 mussel specimens were individually checked for the presence of flatworms, and we didn't find any. A detailed examination of > 3000 mussels weighing about 200 kg (May 2023) used for the preparation of mussel meal and shell powder confirmed that no flatworms were detected in the live mussels (unpublished results). This probably indicates a very short period between the penetration of the flatworm into the mussel and the death of the mussel (opening of the shell).

Table 1
Mussel condition index (meat yield), vitality (LT50), mortality, flatworm occurrence, and *C. oblonga* overgrowth (mean ± SD) at investigated mariculture site in Lim Bay (August 2022–May 2023).

Parameter/Date	August 2022	December 2022	May 2023
Meat yield (%)	18.12 ± 1.69[a]	19.59 ± 1.84	19.09 ± 0.38
LT50 (days)	4.96[a]	9.96*	8.11*
Fiducial limits 95 %	from 3.34 to 5.71	from 8.22 to 10.78	from 6.83 to 9.63
Mortality (%)	5.0 ± 2.0[a]	20.0 ± 5.0*	29.7 ± 2.5*
Flatworms (mean) (no/40 kg mussels)	1.7 ± 0.6[a] (2, 1, 2)	4.3 ± 0.6* (4, 5, 4)	6.0 ± 1.0* (7, 5, 6)
Overgrowth (%)	80[a,b]	15[b]	20[b]

[a] Initial mussel culture ropes.
[b] Coverage visual approximation.
* Significancy $P < 0.05$ in comparison to August 2022.

3.3. Laboratory determination of flatworm feeding preference and performance

Feeding experiments with mussels (*M. galloprovincialis*) and tunicates (*C. oblonga*) exposed to the flatworm *S. mediterraneus* in laboratory aquaria for four weeks in May and September 2023 showed a clear preference of flatworms for mussels. Comparing the predation of mussels by flatworms with that of ascidians, no predation of ascidians was observed, but four mussels were eaten during the experiment in May and five mussels in September. Under laboratory conditions, the flatworms avoided using hanging ascidian colonies for shelter. Instead, they spent most of their time under or around the mussels and showed negative phototactic behaviour (Teng et al., 2022). Once the first mussel was eaten, it was very difficult or impossible to observe the presence of flatworms without removing the mussels from the tank. These small differences in the feeding performance of *S. mediterraneus* (4 vs 5 mussels eaten) can be explained by the seawater temperatures (21 °C vs 25 °C) and the associated higher metabolism of the flatworms. Our standard protocol for keeping flatworms in captivity involves weekly feeding at high seawater temperatures (> 20 °C) with one mussel (approx. 6–7 cm, 30 g, tissue 5 g w.w.) and for the rest of the year we keep 2–3 live mussels with flatworm tanks (10–15 specimens, 40 L). The increasing presence of flatworms in mariculture in Lim Bay reduces the production performance of mussels (*M. galloprovincialis*) and oysters (*O. edulis*) as it increases the cost of monitoring and cleaning. O′Connor and Newman (2002) described feeding preference as a function of prior food availability (*M. galloprovincialis* vs *Pinctada imbricata*), feeding history, size, and habits of flatworm *Stylochus mcgrathi*. So far, our field observations of flatworms in Lim Bay have not revealed any differences in the feeding preference for mussels or oysters (unpublished results).

3.4. Laboratory predation experiment and mussel VG response

Real-time observation of mussel predation by flatworm *S. mediterraneus* using valve gaping mussel monitor was performed for three days (Fig. 4). As a most suitable presentation of mussel VG behavioural response, we chose a line graph of shell opening (0–100 %) during each day (Fig. 7) and a bar graph of cumulative daily mussel VG percent occurrence (24 h, 9:00–9:00 h) (Fig. 8).

By analysing and comparing the results of VG before and during the exposure of mussels to flatworms, including controls, it was possible to observe the behavioural changes of the exposed mussels (stress) towards the control, the timing of flatworm infestation (mussel penetration), the following response of the mussels during predation, and the time of death of the mussel (approx. 30 h after exposure and 26 h after infestation) (Fig. 7). Immediately after adding the flatworms to the tank with VGMM mussels (Day 2), the mussels closed their shells (VG < 10 %) for approximately 2 h, probably as a result of the flatworms exploring the new environment and searching for shelter (Fig. 7B, i). Until evening (23 h; Day 2), the mussels showed normal filtration behaviour, but the valves closed more frequently until the flatworm entered the mussel (ii). On Day 3, compared to the VG behaviour on Day 1, the mussel showed more frequent valve closure and disturbance and finally closed the valves for about 3 h before they died (iii).

The average time occurrence of control mussel valve gaping shows typical binominal distribution analysed by polynomial regression (Figs. 8B and 9, Day 1). However, it is possible to observe VG changes in the mussel behaviour during exposure to flatworms (Fig. 9 Day 2 and 3) compared to the VG behaviour before exposure (Fig. 9, Day 1) and average VG distribution of control mussels (Fig. 8B). When correlating

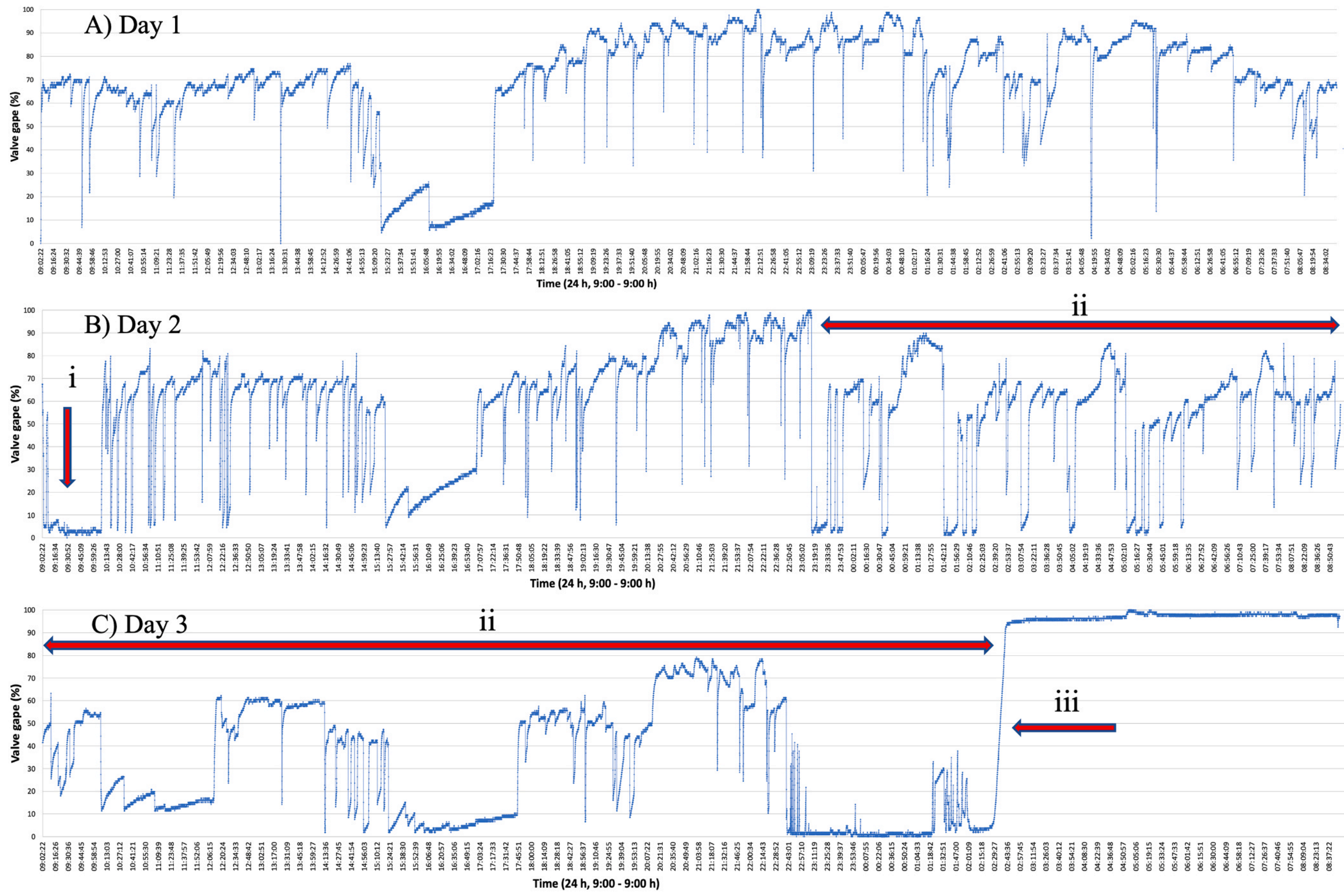

Fig. 7. Observation of mussel predation by flatworm *S. mediterraneus* using valve gaping mussel monitor: A) Day 1, mussel acclimation; B) Day 2, beginning of mussel exposure to flatworm (i); C) Day 3, first attack and mussel response to predation (ii) and mussel death (iii).

the behavioural patterns of the mussels from Days 1 to 3 to the control group, it was found that mussels during Days 1 and 2 were in correlation to the control group ($r = 0.66$, 95 % CI = 0.05–0.91; $P = 0.04$, $r = 0.97$, 95 % CI = 0.86–0.99, $P < 0.001$; respectively), while Day 3 was not ($r = 0.32$, 95 % CI = − 0.38 to 0.79; $P = 0.36$) indicating a different behavioural pattern.

By analysing and comparing the results of VG before (6 mussels) and during predation experiment (2 controls, 2 days), it was possible to observe mussels' VG normal daily rhythm: 69.90 ± 13.40 % of the time filtering with open valve VG > 50 % (high filtration), 27.80 ± 12.75 % VG 31–50 % (low filtration) and 1–5 rest(ing) periods occurrence 27.80 ± 12.75 % and VG occurrence ≤ 30 % (Figs. 7A and 8; Table 2). We also observed that each mussel has its own VG behavioural pattern and repeats the same resting and filtration periods on each day of VG monitoring (Fig. 7A and B). Based on this work and previous unpublished results, we can conclude that the mussel that has more resting periods and less filtration time (VG > 30 %) has better vitality and/or the environmental conditions (food supply) are more favourable. The valve closures (resting) were not synchronised among the six mussels, suggesting that they were not governed by some common stressor. Cameau et al. (2018) described similar normal *M. galloprovincialis* valve gaping behaviour, with maximum valve gape opening at night and a minimum opening during the daytime. They show that mussel aquaculture rafts in Galician favourable waters closes valves rarely (2.55 % daily occurrence) over a 10-day VG monitoring period. The normal VG behaviour with diurnal circadian pattern described in this study for *M. galloprovincialis* corresponds to the general behaviour of filter feeding bivalves in optimal and undisturbed conditions. It is known that this behaviour is related to the relaxing of the adductor mussel and thus to passive (low energy cost) opening of valves (Ait Fdil et al., 2006).

The mussel valve movement (gaping) is widely recognised as a powerful integrative measure of physiological functions such as respiration, feeding, and excretion, which can change under stressful conditions and as a defence reaction to external stimuli (Kramer et al., 1989; Hubert et al., 2022, 2023). Even though the measurements of mussel valve gaping with different methods have received much focus, many authors demand extensive effort to develop advanced data processing and interpretation to ameliorate the quality of the threshold of disturbance of environmental stressors (Bae and Park, 2014; Beggel and Geist, 2015; Redmond et al., 2017; Tonk et al., 2023). Overall, the VG mussel's response appears to be graded, with useful results, but complex to analyse as VGMM generates an extensive data set (Hall sensor reading at minimum measurement frequency 0.5 Hz - every 2 s for 6 mussels/day = 259,200 records). The average temporal occurrence of mussel valve gaping (filtration proxy) shows a typical binomial distribution described by a polynomial regression. However, it is possible to observe VG changes in mussel behaviour (stress) during exposure to flatworms compared to the control and the average distribution of VG occurrence. The VGMM system offers enormous potential for monitoring mussel health, vitality and *in/ex situ* stress response to environmental conditions and as an indicator of normal VG mussel behaviour (filtration function), suitable environmental conditions, extreme conditions (climate change), predation, disease occurrence, including the assessment of toxic phytoplankton blooms (Nagai et al., 2006; Shakspeare et al., 2023).

According to our observations flatworms prefer weaker mussels, but can also feed on healthy mussels, although not all flatworms present, which hide between the mussel culture ropes and overgrowth, attack the mussels at the same time. Only the larger, "experienced" flatworms can attack and kill the mussels, while the others only eat a free meal. No

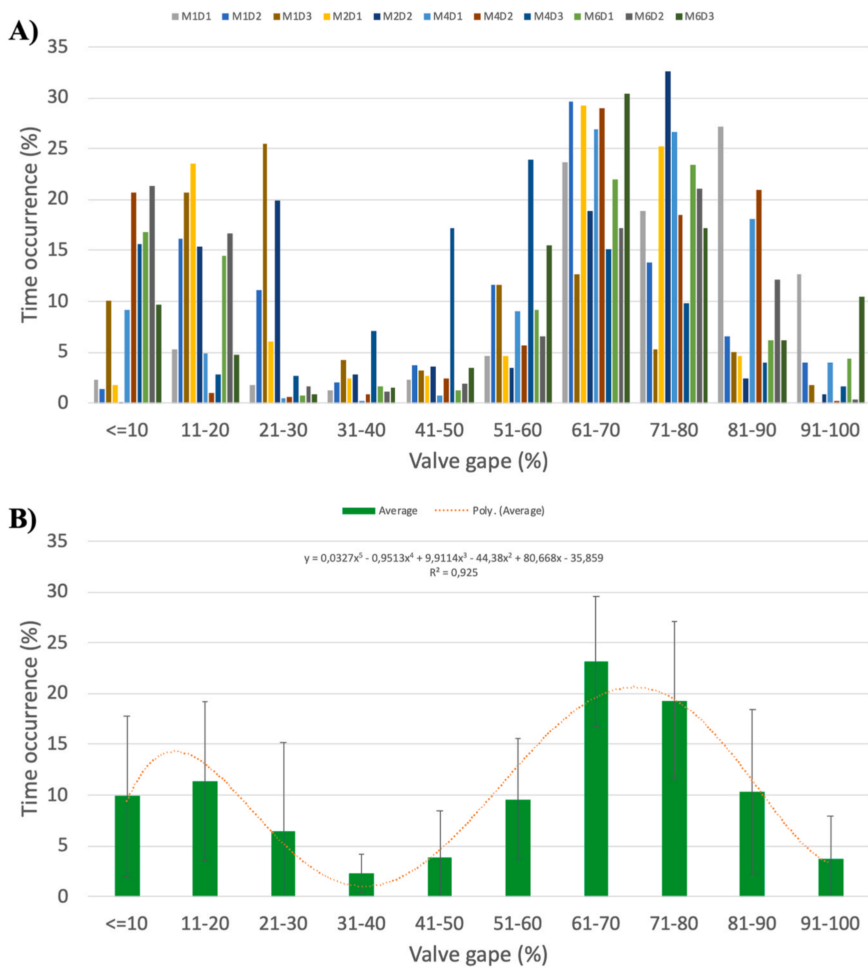

Fig. 8. Percent time occurrence of cumulative daily valve gaping of control mussels (24 h, 9:00–9:00 h): A) MXDY = Mussel no. 1 Day 1 etc., and B) their mean values with standard deviations and polynomial regression.

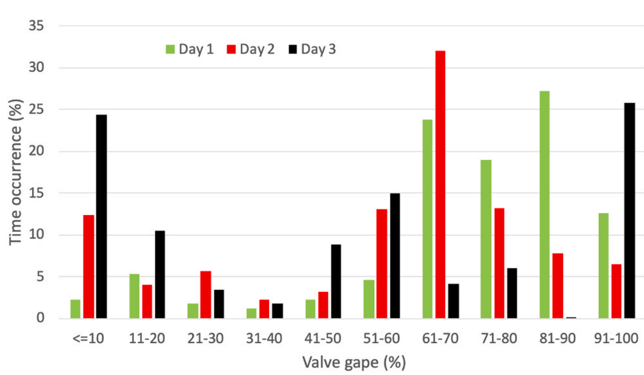

Fig. 9. Percent time occurrence of cumulative daily valve gaping (24 h, 9:00–9:00 h) of mussel attacked by flatworm: Day 1, control; Day 2, beginning of mussel exposure to flatforms, first attack/predation; Day 3, mussel response to predation and mussel death.

Table 2
Results of mussel daily (24 h) valve gaping (VG) time occurrence summarised in three categories: Resting, Low filtration and High filtration.

Mussel VG Occurrence	Resting (0–30 %)		Low filtration (31–50 %)		High filtration (51–100 %)	
	Average	StDev	Average	StDev	Average	StDev
Acclimation (Average)	27.80	12.75	6.17	6.01	69.90	13.40
Day 1 (Control)	9.38		3.50		87.12	
Day 2 (Exposure)	22.00		5.45		72.55	
Day 3 (Exposure)	38.31		10.66		51.03	

flatworms were found in live mussels prepared for sale, as the time between infestation and mussel death is short (approx. 1 day) and dead mussels can serve as a food source for up to a week, depending on the number of flatworms. Nevertheless, numerous predators could pose an additional threat to shellfish farms in the region. Given the slow predation rate and individual prey choice, the potential impact of flatworms in shellfish farms largely depends on the number of flatworms present. Therefore, more frequent cleanings and removal of dead mussels (flatworms) are a possible measure to reduce mortality and production losses.

Following the increase in mussel mortality and the occurrence of flatworms during the study period (August 2022–May 2023) at the investigated site, mussel production in Lim Bay appears to be acutely endangered. The combination of heavy fouling with *C. oblonga* and an increased number of *S. mediterraneus* hiding in the "sods" of the current fouling is a bad combination for the planned increase of local mussel production. The flatworm *S. mediterraneus* is most likely a very opportunistic species, i.e. it takes advantage of a rich food supply resulting from the poor condition of the mussels (vitality) due to fouling by *C. oblonga*.

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CRediT authorship contribution statement

Lea Brumnić: Visualization, Methodology, Formal analysis. **Maja Maurić Maljković:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis. **Andrej Jaklin:** Methodology, Investigation, Funding acquisition, Conceptualization. **Daniele Suman:** Writing – original draft, Methodology, Investigation, Conceptualization. **Srećko Oštir:** Visualization, Software, Methodology, Formal analysis. **Bojan Hamer:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dijana Pavičić-Hamer:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Ivan Balković:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Kristina Grozić:** Formal analysis, Visualization, Writing – review & editing. **Nikola Tanković:** Writing – original draft, Visualization, Supervision, Software, Methodology, Formal analysis, Data curation. **Luca Privileggio:** Writing – original draft, Visualization, Software, Methodology, Formal analysis. **Hrvoje Labura:** Writing – original draft, Methodology, Investigation, Formal analysis. **Matija Hamer:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bojan Hamer reports financial support was provided by Croatian Science Foundation. Bojan Hamer reports financial support was provided by Region of Istria. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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