

*Studies on the effects of short-term exposure to the  
D SP-producing *Prorocentrum lima* on the pumping rate  
of the blue mussel, *Mytilus edulis*, using a new method*

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### Abstract

The influence of short-term exposure to the DST-containing *Prorocentrum lima* on the feeding activity of blue mussels, *Mytilus edulis*, previously exposed to DST's is studied. A new method for measuring pumping activity, also useful for recording heartbeat rate, of mussels is presented.

The mussels showed no response in pumping activity when they were momentarily exposed to *P. lima*, which implies that mussels do not sense and react to the alga or to DST's in the surrounding water. Two hours of exposure to *P. lima* ( $10^3$  cells·ml<sup>-1</sup>) had no effect on the mean pumping activity of the mussels, while variations in individual pumping rates were larger after *P. lima* was added. This effect may, however, be due to the raised concentrations of algae in the water. The heartbeat frequencies of mussels exposed to *P. lima* for two hours did not differ from frequencies of mussels run in *Isochrysis galbana*. Thus, blue mussels previously exposed to DST's seem to be insensitive to short-term exposure to the DSP-producing *P. lima*.

## 1. Introduction

### 1.1 Background

The physiology of the blue mussel, *Mytilus edulis* L., has been subject to extensive research, much due to the economical value of this bivalve as food and to the use of the mussel in biological monitoring. Mussel farming is a highly productive form of aquaculture, with a vast potential for expansion. At the west coast of Sweden Diarrhetic Shellfish Toxins (DST's), caused by occasionally occurring toxic algal blooms, are a major problem for the mussel industry. More information about the occurrence of these biotoxins, and their uptake and metabolism in mussels, is needed. This knowledge could be applied on the development of methods for large-scale depuration of intoxicated mussels in basins or in natural waters before harvesting.

### 1.2 Feeding in *Mytilus edulis*

*Mytilus edulis* is a suspension feeding lamellibranchiate bivalve that obtains its food by retaining particles from the ambient water. Lateral cilia on the gills pump water through the inhalant siphon into the mantle cavity. Particles kept in suspension by fluid mechanical forces are transported to the mouth along the frontal surfaces of the gill filaments by laminar ciliary currents, and are ingested. When the concentration of suspended particles exceeds the capacity of transporting particles in suspension, the surplus material is captured by mucus strings carried on the ciliary tracts. The mucus-bound particles are transported to the labial palps and ejected as pseudofaeces (Jørgensen 1990). This differs from the mechanism presented by Bayne *et al.* (1976a), according to which all particles are bound to mucus and the amount of food entering the mouth is regulated by the palps.

The blue mussel is capable of a complete retention of organic particles down to 4  $\mu\text{m}$  and a 50 % retention of 1  $\mu\text{m}$  particles (Vahl 1972, Møhlenberg and Riisgård 1978). Three mechanisms of sorting suspended organic particles have been recognized in filter-feeding bivalves, though not in *M. edulis*. Selection may occur on the gills, the labial palps and in the stomach (Shumway *et al.* 1985). Sorting of organic particles from silt has been demonstrated in the blue mussel. There is a close correlation between labial palp size and selection efficiency suggesting that the sorting is carried out on the palps (Kiørboe *et al.* 1980, Kiørboe and Møhlenberg 1981). There is some evidence that the blue mussel can feed selectively in suspensions of different types of microalgae. Lesser *et al.* (1992) showed a selectivity for small microalgae (3-5  $\mu\text{m}$ ), and Ward & Targett (1989) found that pre-ingestive chemical cues from microalgae, particularly epicellular ectocrines, influence selectivity in blue mussels. The blue mussel possesses two known pallial sense organs, the osphradium and the abdominal organ, which could be responsible for testing the inhalant current. The osphradium of molluscs is assumed to be chemosensory. There may also be receptors on the edge of the mantle (White 1937, Thompson & Bayne 1972. Granéli *et al.* (1993) and Olsson *et al.* (1992) reported that *M. edulis* is not particularly selective with respect to either size or species of alga.

Blue mussels show substantial individual variability in shell-gape patterns (Ameyaw-Akumfi & Naylor 1987, Kramer *et al.* 1989) and thereby also in filtering activity. Jørgensen (1960) and Winter (1973) found no correlations between variations and diurnal or tidal periodicity, while Ameyaw-Akumfi and Naylor (1987) obtained indications on weak endogenous circadian rhythmicity of shell-gaping.

The water pumping rate has been shown to increase linearly with an increase in the viscosity of the water, i. e. an increase in temperature, while an increase in beating frequency of the lateral cilia does not have any profound effect. The blue mussel filter pump works with a constant pumping pressure, and seems to lack physiological mechanisms for regulation of the pumping rate (Jørgensen *et al.* 1991). The pumping rate can, however, be controlled mechanically by regulation of the valve gape and extension of mantle edges and siphons. Reduced gaping of the

valves and retraction of siphons and mantle edges result in declined pump pressure by reducing the width between the demibranchs (Jørgensen 1990).

### 1.3 Diarrhetic Shellfish Toxins

Diarrhetic Shellfish Toxins (DST's) are produced by dinoflagellates. Most occasions of DST-intoxication of mussels are caused by species of the genus *Dinophysis* (Dahl and Yndestad 1985 Séchet *et al.* 1990, Edebo *et al.* 1993), but also the benthic genus *Prorocentrum* may cause intoxication of mussels (Murakami *et al.* 1982, Rausch de Traubenberg and Morlaix 1995). The DST's are low molecular, heat resistant, lipophilic polyether toxins. In Western Europe okadaic acid (OA) is the dominating substance, but Dinophysistoxin 1 (DTX-1) has also been detected (Edebo *et al.* 1993).

Most of the OA and DTX-1 are accumulated in the hepatopancreas of the blue mussel (Cohen 1990, Edebo *et al.* 1993). OA and DTX-1 seem to be involved in the same metabolic reactions, but the accumulation of OA and DTX-1 does not respond as a progressive build-up with time. This may result from effects of the toxins on the feeding activity, and from bioconversions of toxins dependent on metabolic activity of the mussels (Pillet *et al.* 1995).

### 1.4 Responses of bivalves to toxic dinoflagellates

The effects of PST (Paralytic Shellfish Toxin)-producing microalgae on the physiology and behaviour of bivalves have been well documented, as reviewed by Gainey & Shumway (1988). Reduced filtration rates and increased shell valve closure are the most common responses, which both result in reduced exposure to the PST-containing algae. Other effects noted are impacts on byssus production, cardiac activity and oxygen consumption, but these may be indirect consequences of the former responses. The effects are species specific with great geographical variations. Increased mortality among mussels previously not exposed to PST-containing algae has been observed. These mussels seem to be more sensitive to intoxication than mussels from areas with recurrent toxic blooms.

Few investigations on the responses of bivalve molluscs to DST-containing algae have been made. Pillet & Houvenaghel (1995) have studied the effects of short-term exposure of *M. edulis* from a DST-free area to the DST-producing alga *Prorocentrum lima*. They found that the filtration rate of the mussels decreased significantly after one hour of exposure to an algal concentration of  $10^6$  cells per liter. The response is suggested to be due to a general physiological stress following toxin accumulation in tissues or to direct effects on the gills of DST in the medium.

To my knowledge, there are no similar studies on mussels from areas with recurrent blooms of DST-containing algae. In this study, I will test whether exposure to the DST-producing alga *Prorocentrum lima* affects the feeding activity of *Mytilus edulis* from an area with periodic intoxications of DST's. The emphasis is put on the following questions: is the water pumping rate of mussels affected (1) by pulses of *P. lima*, i.e. do mussels sense and respond to the toxic alga or DST's in the surrounding water, or (2) by short-term (a few hours) exposure to *P. lima*? A new direct method of continuously measuring the water pumping activity of siphonate bivalves is used.

## 2. Materials and methods

The experiments were performed in a laboratory flow through system in February 1996 at Tjärnö Marine Biological Laboratory.

## 2.1 Mussels

The blue mussels, *Mytilus edulis* L., used in the experiments were collected in late December 1995 from a mussel farm sited at Tjärnö in the northern part of the west coast of Sweden. At the time of sampling the mussels contained okadaic acid (54 µg/100 g meat). The mussels used were 53-74 mm long, all having settled in spring 1994. They were maintained in deep sea water (4-8 °C, 33-34 ‰ PSU) until the end of January. At least 10 days prior to the experiments the mussels were set on a monoalgal diet of *Isochrysis galbana*, an alga commonly used as food in laboratory bivalve cultivation.

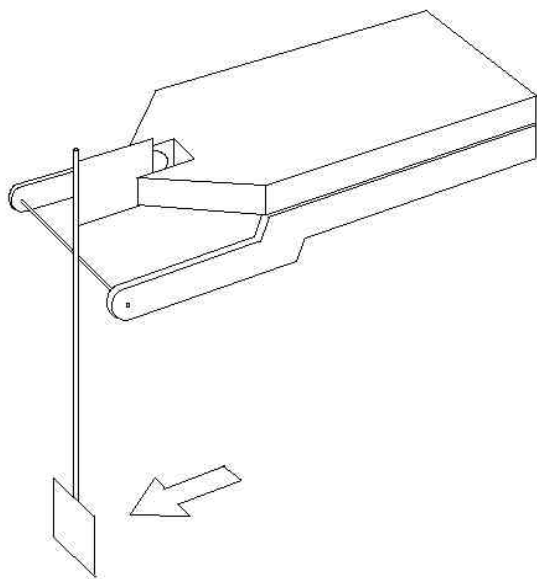
## 2.2 *Prorocentrum lima*

*Prorocentrum lima* is a benthic dinoflagellate, known to produce DST's in culture conditions (Murakami *et al.* 1982). The *P. lima* used in the experiments were cultivated in Medium f/2 at a temperature of 20°C and with a 16:8 h (light:dark) photoperiod. The algae used were in the stationary phase.

## 2.3 Experimental setup

All experiments, except an ingestion experiment, were performed in a flow-through system suitable for testing one mussel at a time. This consisted of a horizontal PVC-tube (length 140 cm, inner diameter 40 mm) cut open in the upper part, a thermostatted water tank and a pump. The water in the tank was circulated by pumping and air bubbling. The mussel was placed at the downstream end of the tube. In the ingestion experiment the mussels were held in a vessel with air bubbling.

The pumping activity of the mussels was recorded using a light diode and a photocell connected to an amplifier and a writer (Graphtec XY Recorder WX 2400). A vertical arm (75 mm long) with a plate (18x18 mm) on the lower end was placed in front of the exhalant siphon from above, the plate being aligned against the exhalant waterstream. The arm was attached 25 mm from the top so that it could move freely in the direction of the waterstream. On the upper part of the arm a flag was placed between the light diode and the photocell, catching light emitted from the diode in correlation with changes in pumping rate, thereby causing the current produced by the photocell to alter (fig. 1). By amplifying the signal the heartbeat frequency of the mussels could be registered.



*Fig. 1.* Apparatus for measuring the pumping activity of mussels. Light emitted from a light diode is caught by the flag in correlation with changes in the velocity of the exhalant current. Changes in light intensity are recorded by a photocell. The signal is amplified and transferred to the writer. The arrow indicates the direction of the exhalant current.

As the experimental setup allowed recording of the water pumping rate of one mussel at a time, findings are based on individual behaviours.

## 2.4 Experiments

In the experiments 15 l (pulse treatments) and 10 l (short-term treatments, test of ingestion) of filtered (0,2  $\mu\text{m}$  filter) sea water with suspended *Isochrysis galbana* cells at initial concentrations of  $5\text{-}8 \cdot 10^4$  cells $\cdot\text{ml}^{-1}$  was used. These concentrations allow continuous filtration (Thompson and Bayne 1972) at optimal rates (Tenore and Dunstan 1973, Foster-Smith 1975). The temperature of the water in the experiments was kept at 6.5-9.5  $^{\circ}\text{C}$ , not varying more than 1.0  $^{\circ}\text{C}$  in single experiments. The salinity was 33-34 ‰ PSU and the flow velocity 90-100 ml $\cdot\text{min}^{-1}$ .

### 2.4.1 Transient exposure

In order to determine whether the mussels sense and respond to the presence of *P. lima* or OA in the surrounding water the effect of pulses of *P. lima* was tested. The mussels were allowed to acclimate to the flow-through system for at least one hour, until they showed steady pumping rates. A dose of 10 ml  $5 \cdot 10^4$  cells $\cdot\text{ml}^{-1}$  of *P. lima* was pipetted into the water 30 cm upstreams of the mussel and the pumping rate before and after the front of the algae reached the mussel was recorded. Tests with a colour indicator (Evans blue) showed that most of the pipetted volume passed the mussel in three minutes. When accounting for a flow rate of 100 ml $\cdot\text{min}^{-1}$  a dilution factor of approximately 1:30 is obtained, if assuming the same pattern for *P. lima*. This means that the mussel was exposed to an average concentration of  $1,7 \cdot 10^3$  cells $\cdot\text{ml}^{-1}$  of *P. lima* during the three-minute period. As a negative respective a positive control a pulse of 10 ml of *I. galbana* of the water in the flow-through system and 10 ml of sea water with the indicator substance methyl violet (ca 0.05 % w/v; an average of 0.0017 % as diluted in flow-through water) were tested. Preliminary experiments had shown that the mussels were able to sense low concentrations of methyl violet in the surrounding water. The number of replicate experiments was five to six.

### 2.4.2 Continuous exposure

In a second experiment, mussels were exposed to *P. lima* for two hours in order to study how the pumping rates of the mussels are affected by a longer time of exposure. The mussels were acclimated to the experimental conditions for an hour, whereafter the pumping rate of the mussel in the suspension of *I. galbana* was recorded for one hour. The cell concentration of *P. lima* in the

stock culture was counted and the alga was added to the tank to an initial concentration of  $1 \cdot 10^3$  cells·ml<sup>-1</sup>. The pumping rate was recorded for two hours after the addition of the toxic alga. The heartbeat frequency was recorded after two hours of exposure to *P. lima* and was compared to measurements of mussels in a monoalgal suspension of *I. galbana*. The treatment was repeated with four mussels.

#### 2.4.3 Ingestion of *Prorocentrum lima*

In order to see if *M. edulis* actually ingests *P. lima* or if there is some mechanism of rejecting the alga, six mussels were placed in a thermostatted vessel with a suspension of *I. galbana*. Two hours later *P. lima* was added to an initial concentration of  $1 \cdot 10^3$  cells·ml<sup>-1</sup>. After another two hours the contents of the digestive system and the faeces of the mussels were examined in microscope.

### 3. Results

In the experiments, recordings of the water pumping rates of mussels kept in a suspension of *I. galbana* showed that the mussels reached a more or less constant rate in 5-40 minutes after they were moved into the flow-through system (fig. 2).

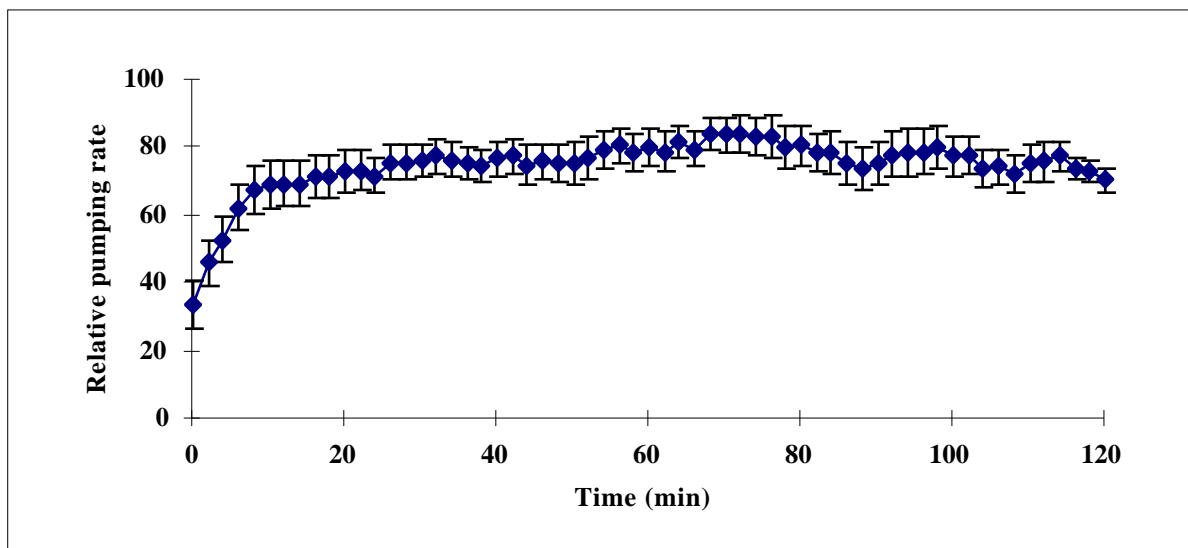


Fig. 2. Mean water pumping rate of *Mytilus edulis* (n = 14) in a suspension of *Isochrysis galbana* ( $5-8 \cdot 10^{-1}$  cells·ml<sup>-1</sup>). Recordings started when the mussel was placed in the flow-through system.

#### 3.1 Transient exposure

No effect of the pulse of *I. galbana* was observed, which indicated that the addition did not disturb the mussels (fig. 3a). Neither did the dose of *P. lima* cause any effect on the pumping activity of the mussels (fig. 3b). The mussels responded strongly to methyl violet in the water (fig. 3c). The reaction was immediate. After about two minutes the mussels started pumping water again; at first for short periods (a few seconds) but the periods were progressively extended until continuous, steady pumping rates were reached after 5-6 minutes.



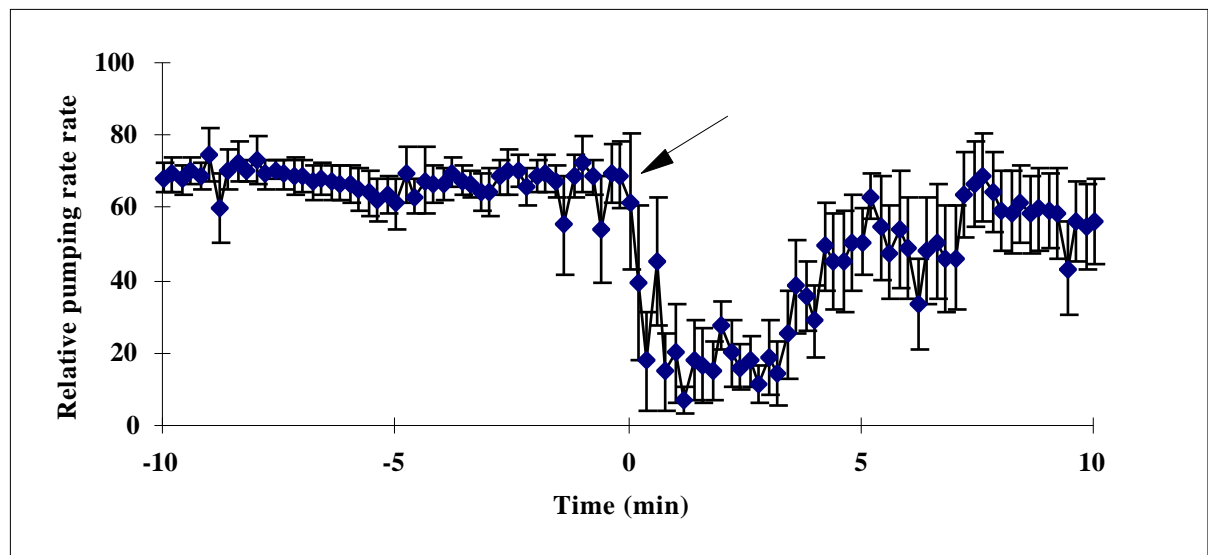
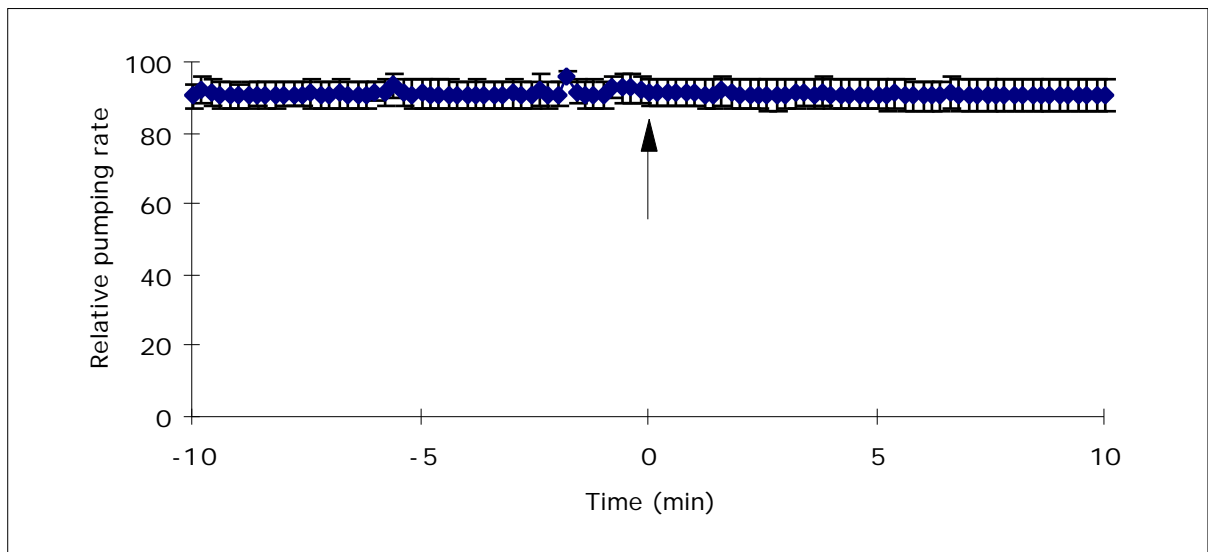
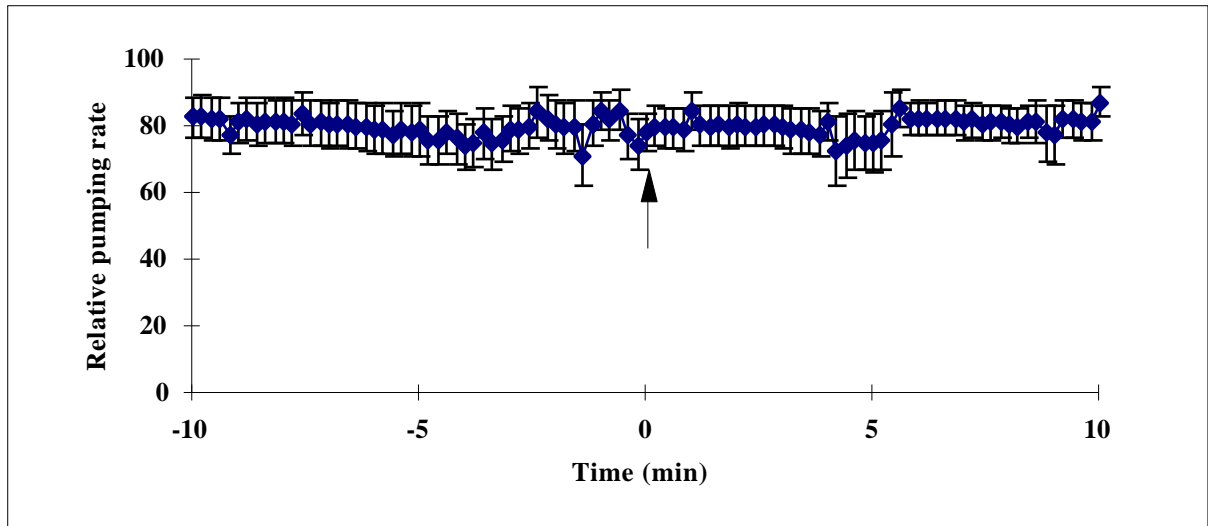


Fig. 3 a-c. Pumping rates (mean  $\pm$  SEM) of *M. edulis* exposed to a pulse of: (a) *I. galbana* (n=6), (b) *P. lima* (n=5), (c) methyl violet (n=5). The arrows show when the front reached the mussel.

### 3.2 Continuous exposure

Exposure to *P. lima* for two hours had no clear effects on the pumping rate. Individual variations in responses were large. The pumping activity of two of the individuals decreased, while the activity of the other two increased. However, means of rates before and after addition were similar (fig. 4). The most obvious effect was that the fluctuations in individual pumping activity became larger after the addition of *P. lima*. The heartbeat frequencies of the mussels exposed to *P. lima* for two hours did not differ from frequencies of mussels run in a monoalgal suspension of *I. galbana* (fig. 5).

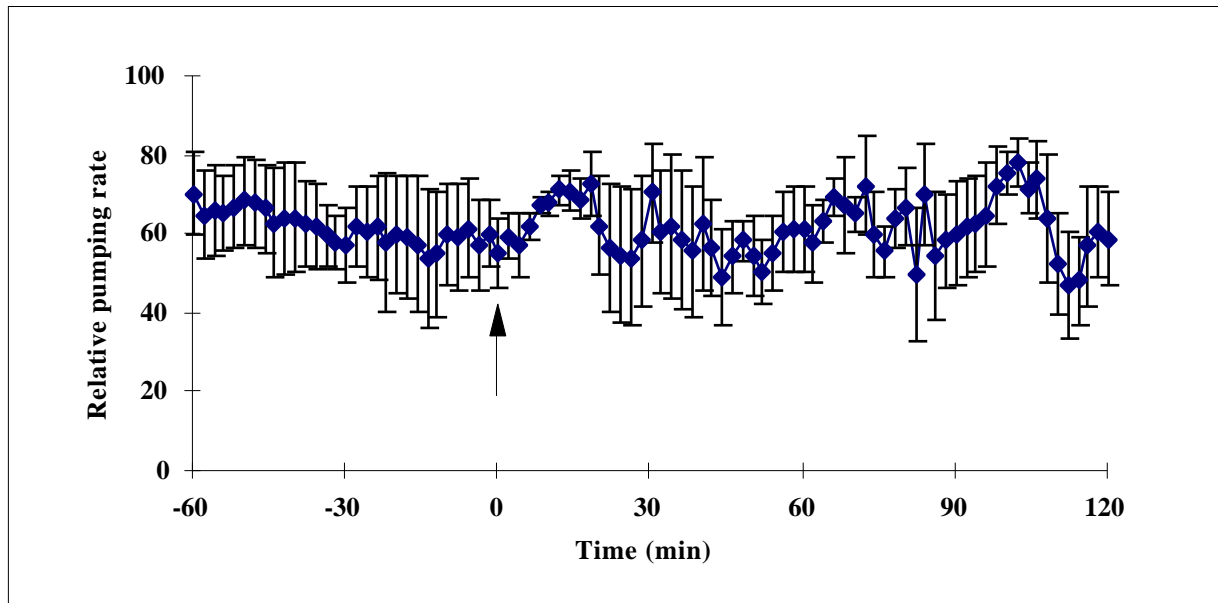


Fig. 4. Pumping rates (mean  $\pm$  SEM) of *M. edulis* exposed to *P. lima* for two hours. The arrow indicates start of exposure.

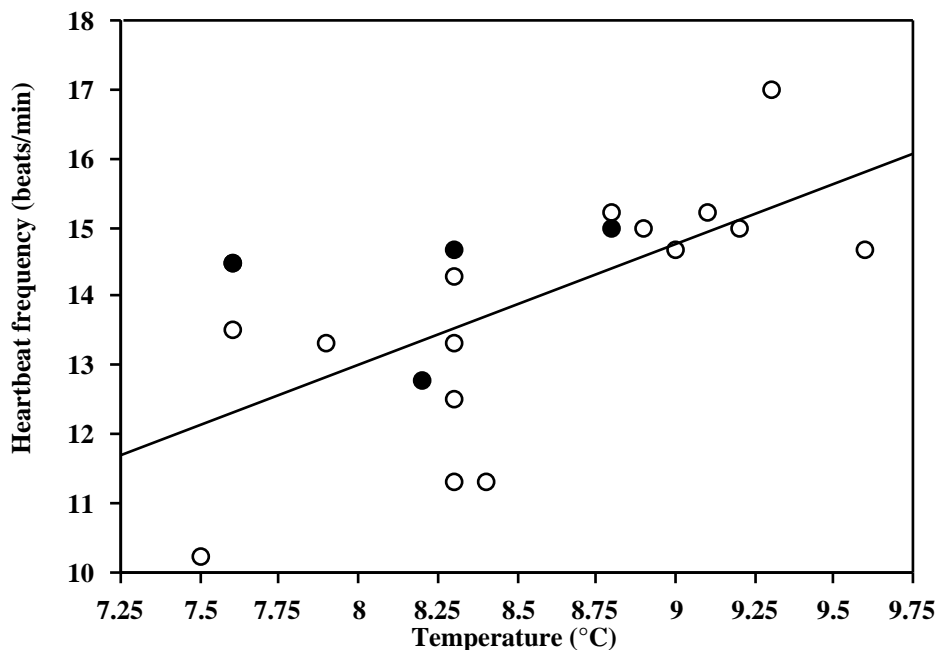


Fig. 5. Regression plot of heartbeat frequencies at different temperatures. Empty circles = individuals run in *I. galbana*, filled circles = individuals run in *P. lima* and *I. galbana* for two hours.

### 3.3 Ingestion of *Prorocentrum lima*

Examinations of the digestive systems of mussels held for two hours in a suspension of *P. lima* revealed that the mussels do ingest *P. lima*. The faeces was not observed to contain any *P. lima* cells, which indicates that the alga had not passed through the digestive system and that no pseudofaeces had been produced.

### Discussion

The method used for continuous recording of water pumping rates is fast and simple. The fact that even heart rates can be recorded indicates that the system is very sensible to changes in pumping rate. Since the method is not fully quantitative, different recordings can not be compared. Some individuals, especially larger ones, seemed to be disturbed by the plate placed in front of the exhalant current. They were able to redirect the current somewhat, with the result that the measured rate was altered. Another factor that can interfere with measurements is that the currents around the mussel in the flow-through system are affected by larger variations in shell-gape. This factor could be excluded if the measurements are made in still water. The mussels used in the experiments did not, however, show much valve movement activity. For smaller mussels (<60-70 mm) the method is adequate, but for large mussels the exhalant current would have to be collected and directed towards the plate to ensure successful measurements.

The relationship between valve gape and pumping rate is ill defined (Jørgensen 1990). It has also been noticed that mussels can react to an adverse environment, e. g. to PST-containing algae, by closing the exhalant siphon, while the valves remain open (Gainey and Shumway 1988). The same behaviour could be observed in the experiments with methyl violet. The instrument used in this work would therefore be a good alternative or complement to measuring shell-gape as the pumping rate is a more sensitive indicator of stress than shell movements.

The heartbeat frequencies of mussels can be measured by amplifying the recorded signal when the pumping activity is high and relatively stable. Fluctuations in pumping rate will, however, make measurements difficult. Methods commonly used require surgery (Bayne *et al.*, 1976b) that will probably disturb the mussel besides being time-consuming. The method tested in present study is fast and produces minimal stress to the mussel, and can thus be an alternative to other methods.

In experiments with pulses of *P. lima*, where mussels were momentarily exposed to the alga, the pumping rates of the mussels were not affected. It has been found that about 20 % of the okadaic acid produced in a culture of *P. lima* is present in the extracellular medium (Rausch de Traubenberg and Morlaix, 1995). Therefore, these results indicate that mussels do not sense and react to OA or to the alga in the medium in rather high concentrations (average conc.  $1.7 \cdot 10^3$  cells·ml<sup>-1</sup>). When exposed to pulses of methyl violet the mussels responded immediately by decreasing the water pumping rate, which shows that *M. edulis* is capable of fast responses to changes in the chemical environment. The response was characterized by a closure of the exhalant siphon and an opening of the edges of the inhalant siphon, in some cases followed by closure of the inhalant siphon and decreased shell-gape.

When exposed to a mix of *I. galbana* (initial conc.  $5-8 \cdot 10^4$  cells·ml<sup>-1</sup>) and *P. lima* (initial conc.  $1 \cdot 10^3$  cells·ml<sup>-1</sup>) for two hours the mean pumping rate of the mussels remained unchanged compared with the rate in the suspension of *I. galbana* alone. The individual pumping rates shifted from being relatively constant before the addition of *P. lima* to changing irregularly. A general increase in pumping activity during the first 20 minutes after the addition was noticed, whereafter no common pattern could be distinguished. Two of the mussels reduced their average pumping activities, while the other two increased their activities. This indicates that there may be individual variations in the responses to the toxic *P. lima*. In order to determine whether the

changes in pumping activity were brought about by exposure to the DST's or if they were caused by the elevated levels of algae in the water a positive control with the non toxic but otherwise similar alga *Prorocentrum micans* would have been needed. Pillet and Houvenaghel (1995) found that the clearance rate of *M. edulis* previously not exposed to toxic algae substantially decreased after one hour of exposure to *P. lima* compared to mussels run in *I. galbana* alone or a mixed diet of *I. galbana* and *P. micans*. This is not the case in the present study, if assuming that the clearance rate and the pumping rate can be compared. Therefore mussels previously exposed to DST's may be less stressed by a diet of DST-containing *P. lima* than individuals from areas free from these toxic blooms. Another possibility is that the mussels from the DST-free area were more susceptible to increased concentrations of algae in the water (see below). Further research will be needed to clarify the possible effects of DST's on the behaviour and physiology of blue mussels from areas with blooms of DSP-producing algae.

The mussels were found to feed continuously at relatively constant paces in the concentrations of *I. galbana* used, which is consistent with earlier findings (Thompson and Bayne 1972, Foster-Smith 1975). At concentrations reached after adding *P. lima* (about 3 mg DW·l<sup>-1</sup>) the mean pumping rate of the mussels did not alter. Rosenberg (1983) has presented a summary of pumping rates at different concentrations of algae in the water obtained in studies made by Vahl (1973), Winter (1973), Riisgård and Møhlenberg (1979) and Riisgård and Randløv (1981). According to this the pumping rate at concentrations similar to that reached after adding *P. lima* would be only 20-25% of that in *I. galbana* alone. The unchanged mean pumping activity could be explained by a delay in the reduction of the feeding activity. The increased concentrations in the mixed suspensions of *P. lima* and *I. galbana* may, however, cause the observed variations in individual pumping rates.

The cardiac activity of mussels run in a mix of *I. galbana* and *P. lima* for two hours did not differ from the frequencies recorded in individuals run in *I. galbana* alone. This agrees with results presented by Thompson and Bayne (1972), who stated that the heartbeat rate is not affected by feeding. Widdows (1973) found a delayed increase in heartbeat frequencies when feeding starved mussels, which indicates that the response is under hormonal control. Accordingly, no immediate effect of DST's can be expected.

These experiments have shown that blue mussels from an area with periodic blooms of DST-producing algae do not respond to a momentary exposure to the DST-producing *P. lima* by altering their pumping activity. Furthermore, the mussels do not seem to be negatively affected by two hours of exposure to the alga, a time sufficient for digestion of *P. lima* to occur. Further research with longer times of exposure to DST's is needed to assess the effects of intoxication. This is an important aspect to consider, as negative effects on the feeding activity or on the physiology of the mussels would indicate that the production in mussel farms can be reduced as a result of toxic blooms.

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