

**Influence of *Labratrema minimus*
(Trematoda: Digenea) on Filtration Rate
Performance of Edible Cockle
Cerastoderma edule in the Extreme
Temperature and Salinity Conditions
(an *in vitro* Experiment)**

A. Javanshir

I.F.R.O., Caspian Sea Ecology Research Academy, P.O.Box: 961 Sari, Iran

E-mail: arashjavanshir@hotmail.com

Abstract: In order to demonstrate filtration rate of edible cockle *Cerastoderma edule* under different salinity and temperature conditions, two groups namely healthy and parasited with *Labratrema minimus* (Bucephallidae; Digenea) were compared. Results showed that the parasited ones had difficulties in adaptation to the extreme conditions of salinity and temperature. Our results also showed that a healthy cockle could filter 78 ml min⁻¹g⁻¹ Flesh Dry Weight (FDW) seawater at 20°C temperature and 34‰ salinity while an infested one processed 15.4 ml min⁻¹g⁻¹ FDW under the same conditions. In low salinity, which could happen during a rainy day and low tide, the healthy cockles processed 14.5 ml min⁻¹g⁻¹ FDW while infested cockles filtered 18.9 ml min⁻¹g⁻¹ FDW. Differences were not significant, however. Filtration rate of healthy cockles at a constant salinity but high temperature was 101.9 ml min⁻¹g⁻¹ FDW while the infested ones rated 27.2 ml min⁻¹g⁻¹ FDW. There was not a significant difference between their filtration rates at 20°C and 30°C. Our results showed that the parasited cockles were unable to adapt to extreme conditions when an increase in temperature or a decrease in salinity occurred. If these results could be extrapolated to natural situations, the ecological cause of mortality due to parasites could be proved and explained.

KEY WORDS: Filtration rate, *Cerastoderma edule*, Digenea, Host-parasite interactions, Himasthla, Labratrema

Introduction

The role of bivalve filtration has received an important consideration in international literature (Jørgensen, 1990 and Beninger *et al.*, 1991). These studies have ended the general conclusion that the bivalves process the water on one hand to feed from phytoplankton and protozoa and on the other hand to insure their oxygen needs and to eliminate the CO₂ produced by the cellular metabolism.

Briefly, in these animals, water enters via the inhalant siphon, circulates in paleal cavity, gets through gills and exits by exhalant siphon. Filtration rate is provoked by ciliary band situated on gills, mantle and visceral mass beating. Valve movements are not directly responsible for water processing, but they could influence water circulation in two situations: (i) at the time when they are closed brutally in order to eject the sand and pseudo-feces, (ii) when they are naturally closed, thus preventing any water circulation via external middle.

About the control mechanisms, Jørgensen (1990) reported that the absence of filtration control related to food capture is consistent with the absence of oxygen supplied. Only works of Massabuau *et al.* (1991) on *Anodonta cygnea* and Tran (1997) on *Corbicula fluminea* suggest that filtration could be closely related to the oxygenation state of water in these animals by means of a very precise control mechanism. Until now, no direct argument in favor of these theories exists for marine mollusks. Concerning other environmental parameters such as temperature or suspended particle concentration, however, these factors were quoted by a certain number of authors as being able to modify the activity of ventilators. Among bivalves, measures of filtration rate and specific oxygen consumption of commercial species, such as mussel and oysters, were the objectives of these research works (Enright *et al.*, 1986; Jones & Allen, 1986; Vismann, 1990; Bernard & Noakes, 1990; Eriksen & Iversen, 1997; and Marsden & Weatherhead, 1998).

The influence of substrate concentration (the proportion of algae-sediment) on food physiology of *Mercenaria mercenaria* (Bricelj & Malouf, 1984) and on mussel (Schulte, 1975) was also studied. Their works showed that filtration rate decreased only when the concentration of algae exceeded $5 \times 10^5 \text{ ml}^{-1}$ in water column. Stenton-Dozey and Brown (1992) also measured

the filtration rate of *Venerupis corrugatus* in connection with the tidal period. Other studies (Wilson, 1984) analyzed the effect of salinity variations on the oxygen consumption of three species: *Cerastoderma edule*, *Macoma balthica* and *Tellina tenuis*. These works brought information on relationship between oxygen consumption and salinity during sharp exhibitions of 24h at 10°C when salinity changes from S=13‰ to 59‰. For these three species, the author concludes that the oxygen consumption decreases only at extreme values of salinity (Fig. 1). Finally, a problem often met in the literature is the variability of results (Table 1) and lack of standardization of the measurements, as well as of units used to express them (debit returned to the dry or fresh weight unit, cockle include or no, or to the unit of size). For example, filtration rate of *C. edule* and *Mytilus edulis* were measured by Willemsen (1952) and expressed solely according to the size of the animals.

So far, relatively few works have been undertaken to elucidate the impact of the parasites on the filtration rate of bivalve. If one considers specifically studies on the effect of parasitic others than the trematods, one can quote Ward (1986) who indicated that ecto-parasitic *Boonea impressa* reduced the filtration rate and growth of oyster *Crassostrea virginica*. In the same manner, the presence of commensally crab *Pinnotheres maculatus* in the inter-valval cavity of mussel *Mytilus edulis* reduces significantly both its filtration rate and oxygen consumption (Bierbaum & Shumway, 1988).

The author's previous studies showed that among other trematods, *Labratrema minimus* was the one that constituted the most important mass (i.e. 50 to 92% of the fresh flesh weight) compared to the body weight of the infested cockle (Javanshir, 1999).

Table 1: Some examples of bivalves' filtration rate variability found in literature (after conversion to: $\text{ml min}^{-1} \text{g}^{-1} \text{FDW}$ unit)

Species (author)	Filtration rate $\text{ml min}^{-1} \text{g}^{-1} \text{FDW}$	Temperature ($^{\circ}\text{C}$)
<i>Arctica islandica</i> (Winter, 1969)	33,333	4
	58,333	10
	68,333	14
	71,667	20
<i>Mytilus edulis</i> (Theede, 1963)	10,333	4
	18,333	10
	22,167	14
	20,667	20
<i>Mytilus edulis</i> (Schulte, 1975)	5,883	5
	24,467	10
	29,183	15
	29,933	20
	29,967	25
	1,766	30
<i>Hiatella arctica</i> (Ali, 1970)	0,009	5
	0,275	10
	0,568	15
	0,201	20

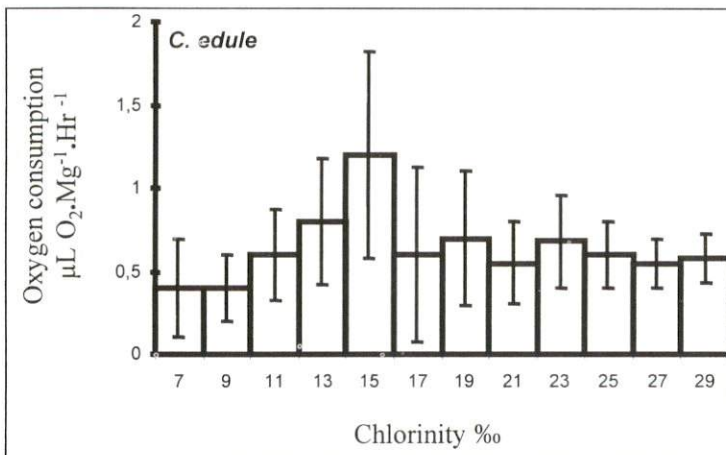


Figure 1: Cockle oxygen consumption variability in different salinities (Wilson, 1984)

As it appeared possible in the first approximation that its impact is also the most important at the individual level, this trematod species was chosen for the present study of parasitism effect on the filtration rate and oxygen consumption. All along our work, adaptation response of parasited animals was systematically studied in the first days following their transfer to the laboratory and compared to those obtained from healthy cockles analyzed in parallel. The idea behind the basis of this step was that in the face of this additional stress, it would be possible to put thus more easily in obviousness of differences between capacities of adaptation of healthy and parasited animals. This is the first time that these kind of studies were conducted at 20°C and in normal seawater. Then, as cockles can be exposed to low tide to strong diminution of salinity of the interstitial water (when it rains for example) and strong increase in temperature during hot days of summer, we have sought whether the parasitism by the trematods could decrease their performance under these conditions. For these reasons, the impact of water was studied where the salinity was decreased to 17‰ and the temperature increased up to 30°C. To our best knowledge, the influence of the parasitic trematods on the filtration rate of bivalves had never been previously studied.

Material and Methods

The filtration rate experiments were conducted in semi-closed circuit in two groups of animals, i.e. healthy and parasited spontaneously by *L. minimus* or infested artificially by *Himasthla spp.* in the laboratory. The following experiments were also performed:

- i. At the time of a Hyposalin shock, an imitation of sea water dilution following strong rain (T = 20°C, S = 34 to 17‰);
- ii. At the time of a temperature increase, an imitation of a sunshiny day during low tide condition (T = 20° to 30°C, S = 34‰); and

- iii. At the time of a Hyposalin shock associated with a brutal temperature decrease.

Preparation of experiment materials

The cockles were sampled from the Pereire site, situated south of Arcachon Bay (France South - West), where previous studies (Javanshir, 1999) showed that other parasite genera infesting the cockles was absent.

- Adaptation: Before any experimentation, the cockles were acclimatized to the laboratory conditions during a period of at least 1 to 2 days. All experiments were run during 3 to 14 days after acclimatizing. The cockles were then installed in an aquarium (25 x 25 x 25 cm) on a 3 cm sand layer, where the temperature varied between 20° and 21°C (natural current sea water temperature).
- Ecto-parasites elimination: Two values of the cockles often shelter between space of a commensally crab species (*Pinotheres* spp.). Since its presence could influence the experiments, the day after cockles' arrival in the laboratory they were posed on a humid surface for 15 min. Their valves were then opened with the help of a plastic pipette. The commensally crab (if present) was then replaced with a pliers. The external surface of cockles was washed to remove the epibionts.

Infested cockles selection:

After their short acclimatizing, they were posed in 250 ml bowls containing seawater. The infested cockles started to drop the cercariae of *L. minimus*. After 24h, all bowls were verified under a magnifying glass; if there was a cercariae deposit, the cockle was infested. Both infested and healthy cockles were then marked before starting the experiments.

Artificial infestation by *Himasthla* spp.

Himasthla utilizes the cockle as its 2nd intermediate host. The first host is the mud snail *Hydrobia ulvae*. For this reason, these snails were taken from the site of Mrennes-Oléron (cart) as the previous studies showed that the

infestation prevalence is high and could reach up to 1% of the population. Just after their arrival in the lab, they were placed in 5 ml seawater and put in a refrigerator at 4-5°C in 6 groups. The day after, they were placed under a lamp where the temperature could reach up to 20 to 25°C. After this thermal shock, the infested snails started to drop the cercariae of *Himasthla* spp. Those having cercariae were selected and divided in two, until reach to determine the infested ones. Totally, 60 infested ones from 500 g snails were collected. From these snails, the cercariae were collected and exposed to healthy cockles.

Infestation protocol

Artificial infestation of the cockles was done in a special Plexiglas tube, closed at two extremities with filets of 1 mm mesh size, allowing the immersion of infested snails near the healthy cockles in the same container. The cockles were exposed to cercariae droppings for 3h in 20°C-seawater.

Algae culture

All animals transported to the laboratory, cockle or snail, were fed with a *Skeletonema costatum* monoculture. This culture was prepared regularly according to Guillard & Rhyter (1962) culture protocols. The final solution distributed to the animals was at a concentration of 10000 to 400 000 algae per ml for 10-liter seawater.

Experiments on the cockles infested by *L. minimus*

These experiments were performed between October 1996 and September 1997 on 49 naturally infested animals. The effects of two levels of salinity, i.e. S = 34 ‰ at 20° and 30°C and S = 17‰ at 20°C were studied. The animals were acclimatized in the laboratory for two days, and the experiments were done 3 to 14 days after their capture. As systematic evolution was not observed during this period, all data was analyzed entirely.

Experiments on the cockles artificially infested by *Himasthla*. spp.

A variety of methods are used for determination of bivalve filtration rate. According to Stenton-Dozey & Brown (1992), these methods could be divided into: 1) direct method (measurement of ventilated volume through the inhalant and exhalant siphons by means of catheters); and 2) indirect method (calculation of the filtration rate and clearance rate of particles ingested by the animal).

In this work, the indirect method was experimented because the techniques are very simple to perform without making any disturbance on siphons thus causing less perturbation on the animal. From this method, disappearance of suspended microscopic algae in a chamber is measured as a function of time.

All infested and healthy cockles were installed individually in the recipients of 250 ml. Thermal adaptation of algae suspension was done in a 5-litre container kept at the desired temperature for each experiment. All recipients of cockle and algae were installed in a thermostatic bath in order to maintain the same temperature. An aerator assured the homogeneity and oxygenation of the recipient.

Calculation method

Measurements were based on the estimation of decrease in microscopic algae concentration during a fix period of time. Jørgensen formula (1990) was used in order to estimate the filtration rate (V_w):

$$V_w = V \times \frac{\text{Ln}(C_{t0}) - \text{Ln}(C_{tn})}{t \times W}$$

where,

V_w : filtration rate ($\text{ml g}^{-1} \text{min}^{-1}$)

V: chamber volume (ml)

C_{t0} : algae concentration at time zero (number per ml)

C_{tn} : final algae concentration (number per ml)

t: experiment duration (min)

W: animal weight, FDW or FFW, (g)

Time laps of each experiment varied based on resistance of cockle in each medium (from 20 to 100 min). Filtration rates were measured as a function of (i): per animal, (ii): per flesh dry weight (FDW), and (iii): per flesh fresh weight (FFW).

When applying the above equation, the following hypotheses should be accepted:

- i. The algal concentration reduction is merely due to the bivalve filtration,
- ii. The filtration rate is constant during the measurement period,
- iii. The retention efficiency is 100%, and
- iv. The algal distribution in the media is homogeneous.

The selection and adaptation period for starving conditions started 48 h before the measurements; the starving period would insure the high particle retention by the cockles. At the end of each experiment, the chamber was examined to assure that no alga was retained on its walls. The short laps of experiment time and the number of replicates had to assume a constant filtration during the experiments. In any case, the experimentation time was shorter than the gut passage time of *C. edule* calculated from Hawkins *et al.*, (1990) formula: $Y = 1.71 X^{0.41 \pm 0.32}$ where Y is in hour and X in g FDW, giving us 68 minutes. The homogeneity of media in the chamber was insured by means of: (i) the current created by the animal, (ii) the current created by input into the chamber, and (iii) the aerators in each recipient.

Algae counting

Counting of algae concentration for each recipient was done by a Counter Coulter (Model ®ZI). The aperture size was ruled at 100 µm. Before counting, the size distribution of algae suspension was determined within classes of 1 to 10 µm. The 4-7 µm size range was selected for counting. The sampler volume was 5 ml. Before each counting, the sample was diluted ten times. Counting was done 5 times; thus, the measurements are an average of 5 counting.

Statistical analysis:

The comparisons of averages were performed by student T test (homogenous variances) or Z test (heterogeneous variances), using Statistica version 5.

Results

The filtration rates observed for healthy cockles were as follow:

Measures at S = 34 ‰ and T = 20°C

Results are presented in Fig. 2 that illustrates the average values, as well as the individual value distribution, determined in these conditions are well reproducible between the two dates. One of the most interesting results is however the great variability of measures since the former varied from 3 to 250 ml min⁻¹g⁻¹ FDW, while values most frequently measured were between 40 and 60 ml min⁻¹g⁻¹ FDW (average=78 ml min⁻¹g⁻¹ FDW, σ =10, n=37). Values expressed in fresh weight correspond to a range of flow rate varying from 0.1 to 34.9 ml min⁻¹g⁻¹ FDW (average = 7.1 ml min⁻¹g⁻¹ FDW, mode = 6-8 ml min⁻¹g⁻¹ FDW, σ = 1.0, n = 37). It is interesting to note that, as for animal of standard size (\approx 30 mm used in these experiments), the fresh flesh weight is approximately 1 g organic matter (without shells); these values correspond equally to the filtration rate means expressed by the animal. A cockle recently transferred to the laboratory would process therefore approximately 10 liters of water per day.

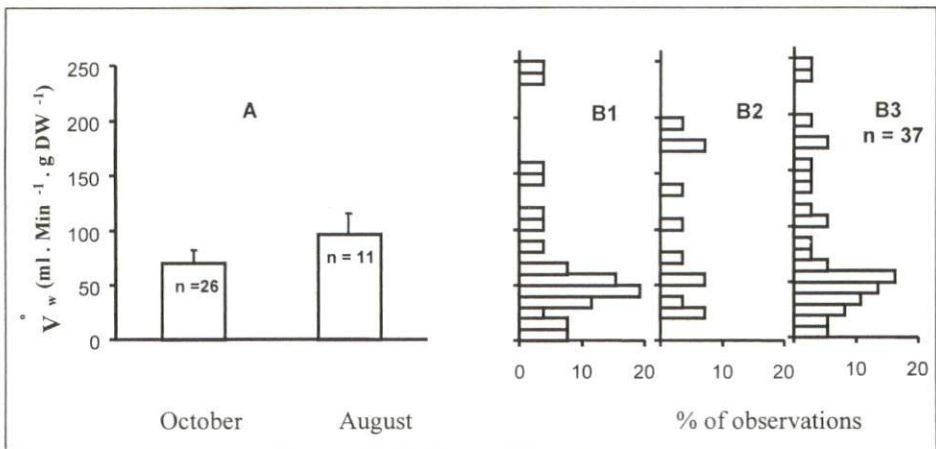


Figure 2:-Filtration rate of healthy cockles at 20°C temperature and 34‰ salinity measured during two periods (October 1996 (B1) and August 1997 (B2)). A: average values with standard errors. There is not any significant difference between the means of two periods. B3 cumulated histograms of all experiments

Measurement at constant salinity (S = 34%) and T=30°C

In nature, during a low tide occurring in a sunny afternoon, temperature of interstitial water can increase entirely in a significant manner. Thirty minutes can be sufficient to observe an increase of approximately 10°C.

The filtration rate of healthy animals facing these types of stress was studied in the laboratory throughout the course of this research. The results are shown in Fig. 3. It was observed that the average value increased up to 101.9 ml min⁻¹g⁻¹ FDW at 30°C (Fig. 3A); but, it is clear that this evolution is mainly due to the weak values disappearance observed at 20°C (Fig. 3B, 3B₂).

The totality of these data at 20 and 30 °C, show capacities of healthy cockle adaptation faced to temperature changes, suggest that the increase in filtration rate at 20°C could correspond mostly to a response to the stress induced by experimental conditions (or to another filtration stimulus) more than to metabolic needs at this temperature.

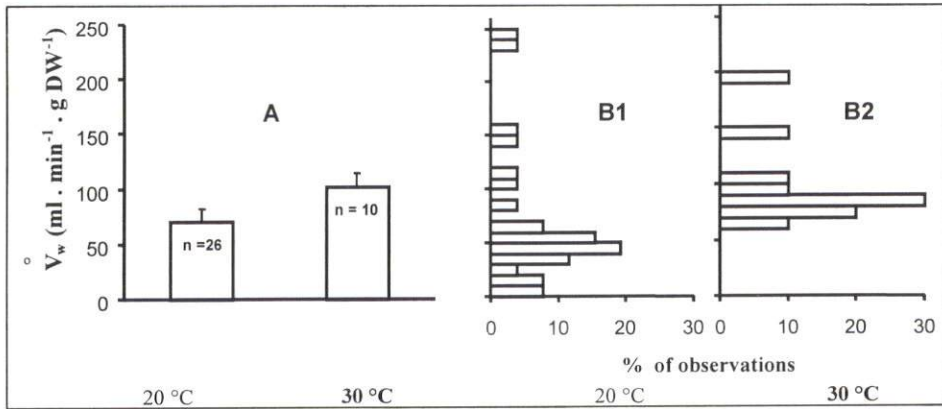


Figure 3: Effect of temperature increase from 20° to 30°C on filtration rate of healthy cockles in a constant salinity of 34%. The average value increased significantly ($z = 2.846 **$). Comparison between Figures B1 and B2 shows that this is essentially due to disappearance of weak filtration rate at 30°C.

Measurement at constant temperature ($T=20^{\circ}\text{C}$) but decreasing salinity from 34% to 17%

The filtration reply observed is shown in Fig. 4, characterized by a significant reduction of average $m = 78 \text{ ml min}^{-1} \text{g}^{-1} \text{FDW}$ ($\sigma = 10$, $n = 26$) to $14.5 \text{ ml min}^{-1} \text{g}^{-1} \text{FDW}$ ($\sigma = 1.6$, $n = 11$).

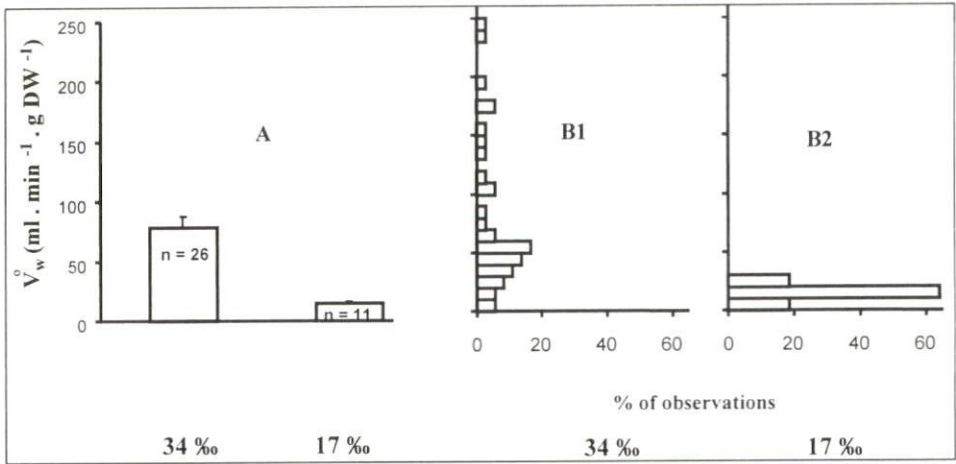


Figure 4 - Effect of salinity decrease from 34 to 17% on filtration rates of the healthy cockles in a constant temperature (20°C). There is a significant difference ($z = 2.412 *$) between the two means. Comparison between B1 and B2 shows that this difference is mainly due to disappearance of high values at 17%.

The filtration rates observed for infested cockles by *L. minimus* Measures at $T = 20^{\circ}\text{C}$ and $S = 34\%$

The filtration rate of naturally infested cockles, measured in laboratory during the first day following their transfer, is presented in Fig. 5 comparing the filtration rates of infested and healthy ones. Values of the filtration rate have been expressed in two manners. They are brought, on one hand to the total mass of organic matter (cockle body + parasite), and on the other hand to the mass of the animal alone (without parasite). Whichever is the mode of expression, average values (total mass, animal + parasite: $V_w = 15.4 \text{ ml min}^{-1} \text{g}^{-1} \text{FDW}$, $\sigma = 1.6$, or animal alone: $V_w = 29.5 \text{ ml min}^{-1} \text{g}^{-1} \text{FDW}$, $\sigma = 2.8$; $n = 28$) of filtration rates were significantly lower than those of healthy ones (Fig. 5A). The individual value distribution showed a very weak variability of

data that are all grouped in the low range between 1.5 to 31 ml min⁻¹g⁻¹ FDW.

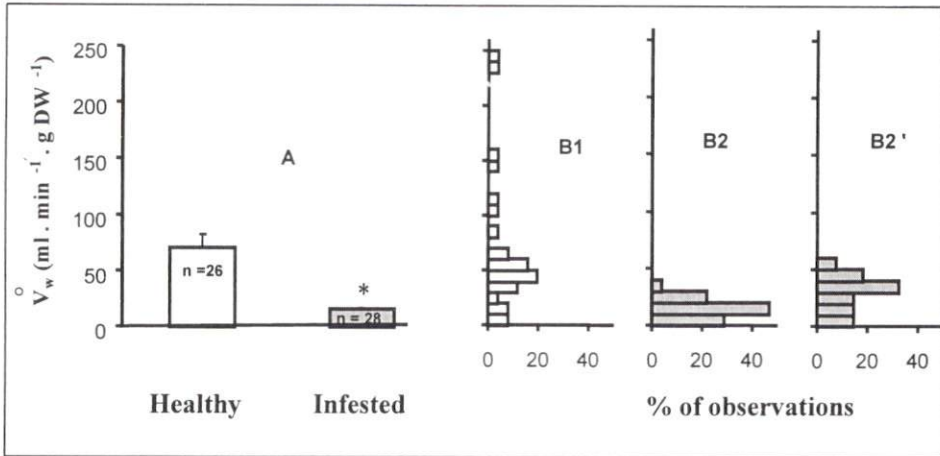


Figure 5: Comparison between filtration rates of healthy cockles (white columns) and infested ones (gray columns) at 20°C and 34‰. Filtration rate is smaller and similar in infested ones ($z = 4.118$ ***). Section B2' shows also the specific filtration rates of infested cockles without their parasite (divided by pure cockle weight). The mode resembles that of healthy ones.

Measurement at constant salinity (S = 34‰) but an increasing temperature from 20° to 30°C

Fig. 6A shows increase in filtration rate observed in healthy individuals while for the infested ones, there was no significant increase in filtration rate (at 20°C: 15.4 ml min⁻¹g⁻¹ FDW, $\sigma = 1.6$, n = 28 ; at 30°C: 27.2 ml min⁻¹g⁻¹ FDW, $\sigma = 4.4$, n = 10). Also, as in the case of 20°C and 34 ‰, the distribution of values stayed very low for infested cockles (5 to 50 ml min⁻¹g⁻¹ FDW) while the healthy ones processed the water at high range of values (60 to 200 ml min⁻¹g⁻¹ FDW).

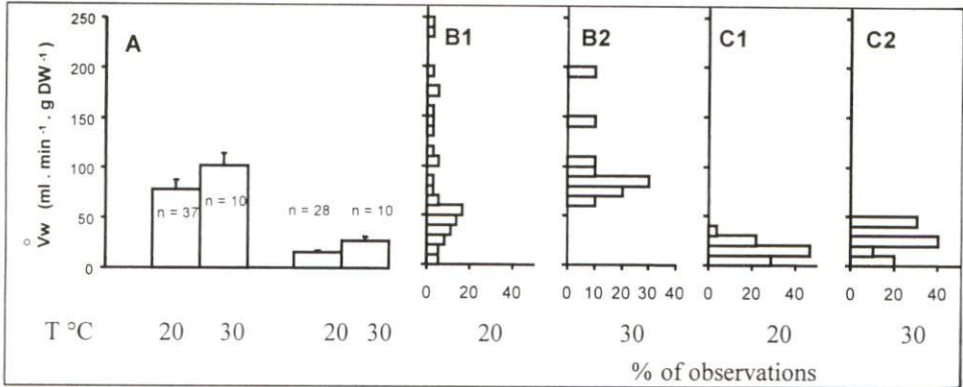


Figure 6: Comparison between filtration rates of healthy cockles (white columns) and infested ones (gray columns) at two temperature (20° and 30°C) and 34% salinity conditions on right. On left, value distributions of each right hand columns (B1, B2) for healthy cockles and (C1, C2) for infested ones are shown.

Measurement at constant temperature (T = 20°C) but decrease in salinity from 34 to 17%

These results are presented in Fig. 7 with the filtration rate of the healthy cockles as a reference on the left part. Here also, the absence of any significant changes in filtration rate of infested animals could be observed (at 34%: 15.4 ml min⁻¹g⁻¹FDW, $\sigma = 1.6$, n = 28; at 17%: 18.9 ml min⁻¹g⁻¹FDW, $\sigma = 2.5$, n = 11) compared to those of healthy ones. Nevertheless, it is necessary to note that these values are rigorously identical to the filtration rate expressed by a healthy cockles responding to a decline in salinity as imposed in this study.

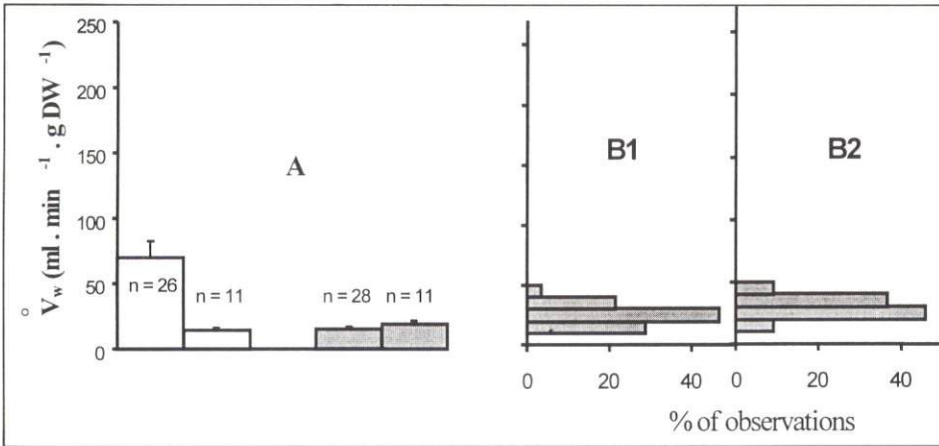


Figure 7: Comparison between filtration rates of healthy cockles (white columns) and those infested by *L. minimus* (grey columns), when salinity decreased from 34 to 17%. B1 and B2 are filtration rates of infested cockles. There was not any significant change between their responses at two different salinities.

Measure when salinity decreased from 34 to 17% and temperature increased from 20° to 30°C

The temperature was increased from 20° to 30°C in 2 h. The animals were then transferred brutally in seawater where the salinity decreased to 17% (at 30°C). The analysis has in fact simply consisted of counting the mortality rate in two populations because at the end of 20 min exposure, 50% of the infested animals (5/10) died (state characterized by a relaxation of the state of closing down of their valves). The total duration of the experiment was 1 hour and no mortality was observed in safety group. To note more than animals of this group have remained totally closed during exhibition and no filtration activity was made.

Filtration rate of cockles infested artificially by *Himasthla* spp.

The effect of this infestation is illustrated in Fig. 8. These results show that they are not much different from those infested by *L. minimus*. The filtration rate in the infested group was: $24.1 \text{ ml min}^{-1} \text{ g}^{-1} \text{ FDW}$ ($\sigma = 2.9$, $n = 25$) beside $77.7 \text{ ml min}^{-1} \text{ g}^{-1} \text{ FDW}$ ($\sigma = 10.4$) Fig. 7 and 8.

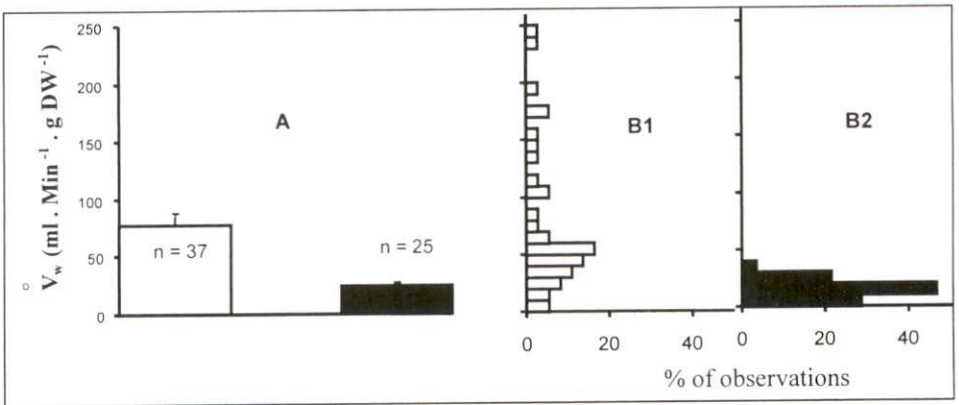


Figure 8: Comparison between filtration rates of healthy cockles (white columns) and cockles artificially infested by *Himasthla* spp. (black and white columns). Similar to the case of *L. minimus*, the filtration rate decreased without any variability. Experimental conditions were temperature of 20°C and 34% salinity.

Discussion

In this work, respiratory physiology was used as a tool to understand some aspects the ecological impact of the parasitism by Trematods on the cockles. Particular attention was paid to the aerobic metabolism relationship, filtration rate-parasitism, an issue that had never been approached in the literature to the best of our knowledge.

All experiments were performed on animals freshly transferred to the laboratory within 1 to 2 days. The idea was to use the stress associated with

this transfer as a tool, possibly aggravating potential differences between parasited and healthy animals in control conditions and when faced with temperature and saltness changes.

The stress following capture and transport was studied as a factor for aggravating differences between filtration rates of healthy and infested cockles. The stress is indeed a very strong ventilation stimulus in aquatic animals (McMahon, 1985; Truchot, 1992; Legeay & Massabuau, 1999). From this study, it can be concluded that the increase in filtration rate of healthy animal could be a reflection, sensitivity diminution, or lack of reactivity of infested ones facing laboratory conditions. These results also showed that:

- During an increase in temperature from 20° to 30°C, cockles infested by *Labratrema* appeared incapable of increasing their filtration rate unlike what one observes in the healthy animals. Therefore, it is likely that under these conditions, the infested animal be incapable of increasing its oxygen consumption too. This hypothesis remains however to be verified.
- During a diminution of salinity from 34 to 17‰, cockles infested by *Labratrema* were not capable of decreasing their filtration rate as compared to a reference state. Nevertheless, it should be noted that the reference filtration rate was, before all change of salinity, already same as which, ends a healthy animal in the same conditions.
- During an increase of temperature from 20° to 30°C and an associated salinity diminution from 34 to 17‰, 50% of infested cockles died in less than an hour.

The parasitism by *Labratrema* would therefore carry, in addition to weight reduction, a diminution of reactivity when compared to the healthy animals facing such stress as exposure to laboratory conditions and an absence of hyper-ventilation capacity during an increase of temperature. The totality of these stress, associate to a diminution of salinity, pull the death.

It therefore became clear that the parasitism associated with *Labratrema* implies a severe diminution of adaptation capabilities by cockles to different environmental parameters, such as temperature and salinity.

Experimental condition criticism:

When an aquatic animal is transferred to the laboratory from its natural habitat, it goes through an adaptation stage that can vary from several days to several weeks before reaching a possible stationary state if adapting conditions

are satisfying (Massabuau & Forgue, 1996). From an experimental point of view, there exists a basic problem concerning the closest state to which prevailed in nature before all intervention. In this subject two opinions confront: on one hand, partisans of the absence of adaptation or more exactly a restricted adaptation; on the other hand, those that oblige a long term adaptation varying from some days to several weeks. Partisans of the first opinion notice that from the sampling site and after arrival to the laboratory, the physiology of animals is upset by these new conditions that restabish only bit similar to the natural environment. It would be therefore necessary to work as fast as possible to be the most ready possible the original state. Partisans of waiting until a balance state think that the adaptation period is a period that does not correspond to that provided in nature (by definition, it is a transition phase), while the whole phenomenon is particularly difficult to interpret. They also consider that the balance phase can indeed be totally different from natural conditions. However, the main favor is that a certain number of analyses, concerning intrinsic adaptation capabilities of a species, then become far simpler to analyze. This approach can not however concentrate on the study of such phenomena as biological rhythms linked to the alternation of tides that are difficult to do in laboratory. This was the reason why the first approach was chosen, i.e. the second approach was technically unable to reproduce the tidal rhythm in laboratory. Under the experimental conditions employed in this study, it therefore represents an important or extreme stress state for cockles as this stress state could certainly aggravate the responses of filtration rate at the extreme conditions. In this work, however, it was decided to use this approach in order to elucidate the action of parasitism.

Application to natural conditions

In nature, populations of *Cerastoderma edule* are dispersed in intertidal zone on all coasts of Europe from Norway to Portugal. This zone is exposed to temperature changes and to seawater salinity. Survival of these animals in this zone would thus depend partly on capacities of resistance against abrupt changes in their environment. For instance, these animals can be exposed to a sharp sun shine during low tide in summer.

In this study, it appeared that healthy shells, facing with temperature and salinity variations, could deeply modify their filtration rate activity in order to adapt to the new conditions and to insure possible changes in corresponding oxygen consumption. This increase in filtration rate is associated, on one hand, with an increase in animal metabolism, and on the other hand,

potentially, with a decline of oxygen concentration dissolved in water when the temperature increases. Works of Bayne (1971) and Bayne *et al.* (1987) on *Mytilus edulis* mussel suggest that an increase in ventilation is a mechanism that compensates the increase in oxygen needs under these conditions. Comparable studies by Jørgensen (1990) show that, in this bivalve, the filtration rate increases when water temperature rises. This author reported a doubled filtration rate for a change in temperature from 8° to 20°C, while it was proved in this study that filtration rate increased 60 %, on average, when temperature increased from 20° to 30 °C. Jørgensen (1990) noted that increase of filtration rate is equally correlated with decline of water kinetic viscosity when temperature increases.

Furthermore, our results showed that the filtration rate of healthy cockles decreased when the salinity declined from 34 to 17%. The most reasonable explanation is that this diminution of filtration corresponds to limited exposure to water, and therefore a problem of osmotic limitation. Works of Wilson (1984) suggest very strongly that in these conditions, the cockle maintains constant oxygen consumption until 17% salinity. Indeed, this author compared aerobic metabolism values with filtration activity in three Lamellibranches species (*Tellina tenuis*, *Macoma balthica* and *Cerastoderma edule*) as water salinity changes. The same author reported that *C. edule* alone maintains its valves open when the salinity changes. The results of this study are in agreement with the above statement that cockles did not close their valves in 17% salinity water. In parasited cockles, filtration reaction was not observed; but, it is remarkable to observe that the absolute value of filtration is already in reference state, i.e. identical to the filtration rate of healthy cockles.

Under these conditions in nature, lack of filtration adaptation in infested cockles would not therefore interfere with its acclimatization capacities. Finally, this study on rapid cockle infestation by exposure to *Himasthla* spp. showed that the effects of parasitism can develop very rapidly. However, it seems this set of experiments were conducted under conditions of infestation that probably was artificially massive as compared to real situations in nature; nevertheless, it suggests that the parasite can potentially interfere rapidly with normal physiology of *Cerastoderma edule*.

Acknowledgement

I am grateful to Dr. J.C. Massabuau from LEESA laboratories of Arcachon, for his helps with these experiments.

References

- Bernard, F.R. and Noakes, D.J. , 1990. Pumping rates, water pressures, and oxygen use in eight species of marine bivalve molluscs from British Columbia. *Can. J. Fish. Aquat. Sci.* **47**:1302-1306.
- Bayne, B.L. , 1971. Oxygen consumption by three species of lamellibranch molluscs in declining ambient oxygen tension. *Comp. Biochem. Physiol.*, **40A**, 955-970.
- Bayne B.L. ; Hawkins, A.J.S. and Navarro, E. , 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixture of silt and algal cells at low concentrations. *J. Exp. Mar. Biol. Ecol.*, Vol. **111**, pp.1-22.
- Beninger, P.G. ; Le Pennec, M. and Donval, A. , 1991. Mode of particle ingestion in five species of suspension-feeding bivalve molluscs. *Mar. Biol.*, **108**:255-261.
- Bierbaum, R. and Shumway, S.E , 1988. Filtration and oxygen consumption in mussels *Mytilus edulis*, with and without pea crabs, *Pinnotheres maculatus*. *Estuaries*, **11**:264-271.
- Bricelj, V.M. and Malouf, R.E. , 1984. Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. *Mar. Biol.*, **84**:155-165.
- Enright, C.T. ; Newkirk, G.F. ; Craigie, J.S. and Castell, J.D. , 1986. Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros gracilis* Schütt of varied chemical composition. *J. Exp. Mar. Biol. Ecol.*, **96**:15-26.
- Eriksen, N.T. and Iversen, J.J.L. , 1997. On-line determination of respiration rates of aquatic organisms in a mono-phase oxystat at steady-state dissolved oxygen tension. *Mar. Biol.*, **128**:181-189.
- Guillard, R.R.L. and Rhyter, J.H. , 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve). *Can. J. Microbiol.*, **8**:229-239.
- Hawkins, A.J.S. ; Navarro, E. and Iglesias, J.I P. , 1990. Comparative allometries of gut-passage time, gut content and metabolic faecal loss in *Mytilus edulis* and *Cerastoderma edule*. *Mar. Biol.* **105**: 197-204.

- Javanshir, A. , 1999. Effets de quelques parasites (Digenea) sur la dynamique des populations et la physiologie respiratoire de la coque *Cerastoderma edule* (Mollusque Bivalve) dans le bassin d'Arcachon. Thèse de doctorat de l'Université Paris 6, 102 P.
- Jones, H.D. and Allen, J.R. , 1986. Inhalant and exhalant pressures in *Mytilus edulis* and *Cerastoderma edule*. J. Exp. Biol. Ecol., **98**:231-240.
- Jørgensen, C.B. , 1990. *Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology*. Olsen & Olsen, Fredensborg, 140 P.
- Legeay, A. and Massabuau, J.C. , 1999. Blood oxygen requirements in resting crab *Carcinus maenas* 24 hours after feeding. Can. J. Zool. (sous presse).
- McMahon, B.R. , 1985. Functions and functioning of crustacean hemocyanin. *In: Respiratory pigments in animals. Relation structure function*. (eds. J.N. Lamy; J.P. Truchot and R. Gilles). Springer, New York, pp.35-58.
- Marsden, I.D. and Weatherhead, M.A. , 1998. Effects of aerial exposure on oxygen consumption by the New Zealand mussel *Perna canaliculus* (Gmelin, 1791) from an intertidal habitat. J. Exp. Mar. Biol. Ecol., **230**:15-29.
- Massabuau, J.C. ; Burtin, B. and Wheathly, M. , 1991. How is O₂ consumption maintained independent of ambient oxygen in mussel *Anodonta cygnea*?. Respiration Physiology, **83**:103-114.
- Massabuau, J.C. and Forgue, J. , 1996. A field vs laboratory study of blood O₂-status in normoxic crabs at different temperatures. Can. J. Zool., **74**:423-430.
- Schulte, E.H. , 1975. Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. Mar. Biol., **30**:331-341.
- Stenton-Dozey, J.M.E. and Brown, A.C. , 1992. Clearance and retention efficiency of natural suspended particles by the rock-pool bivalve *Venerupis corrugatus* in relation to tidal availability. Mar. Ecol. Prog. Ser., **82**:175-186.
- Tran, D. , 1997. Influence de l'oxygénation de l'eau et de l'activité respiratoire sur les mécanismes de bioaccumulation du cadmium chez le bivalve d'eau douce *Corbicula fluminea*. DEA Université Bordeaux 1, 23 P.

- Truchot, J.P. , 1992. Respiratory function of arthropod hemocyanins. *In* : Advances in Comparative and Environmental Physiology, Vol 13. (ed. C.P. Mangum), Springer, Heidelberg, pp.378-410.
- Vismann, B. , 1990. Field measurements of filtration and respiration rates in *Mytilus edulis* L.: an assessment of methods. *Sarsia*, **75**:213-216
- Ward, J.E. , 1986. Effects of the ectoparasite *Boonea (Odostomia) impressa* (Say) (Gastropoda: Pyramidellidae) on the growth rate, filtration rate and valve movements of the host *Crassostrea virginica* (Gmelin). *J. Exp. Mar. Biol. Ecol.*, **99**:163-180.
- Willemsen, J. , 1952. Quantities of water pumped by mussels *Mytilus edulis* and cockles *Cardium edule*. *Arch. Néerl. Zool.*, **10**:153-159.
- Wilson, J.G. , 1984. Assessment of the effect of short term salinity changes on the acute oxygen consumption of *Cerastoderma edule*, *Macoma balthica* and *Tellina tenuis* from Dublin Bay. *J. Life Sci. R. Dubl. Soc.*, **5**:57-63.