

COMMENT

Thermal and viscous effects on ciliary suspension-feeding bivalves — no need for a new explanation: Comment on Specht & Fuchs (2018)

Hans Ulrik Riisgård^{1,*}, Poul S. Larsen²

¹Marine Biological Research Centre, University of Southern Denmark, Hindsholmvej 11, 5300 Kerteminde, Denmark

²DTU Mechanical Engineering, Fluid Mechanics, Technical University of Denmark, Building 403, 2800 Kgs. Lyngby, Denmark

ABSTRACT: In a recent paper, Specht & Fuchs (2018; Mar Ecol Prog Ser 589:129–140) claim that 2 ciliary suspension-feeding bivalves, the blue mussel *Mytilus edulis* and the hard clam *Mercenaria mercenaria*, differ in how they respond to viscosity. For *M. mercenaria*, the authors found no change in filtration rate or beat frequency of water-pumping cilia in response to changes in viscosity at constant temperature. For *M. edulis* on the contrary, a previous study found these parameters to depend on manipulated viscosity at constant temperature in the same way as viscosity changes with temperature. To reconcile the opposing views, Specht & Fuchs suggested that the 2 bivalves may fundamentally differ in their responses to viscosity. But this suggestion is unwarranted. In addition to other shortcomings, we show for example that Specht & Fuchs likely misidentified compound laterofrontal cirri as lateral cilia, leading to erroneous conclusions. Furthermore, general fluid mechanical aspects were not considered by Specht & Fuchs, although many studies have shown that temperature-dependent viscosity of the ambient water controls or strongly affects bio-mechanical activity, such as beat frequency of water-pumping cilia in suspension-feeding bivalves, as well as water flows in general.

KEY WORDS: Viscosity · Temperature · Lateral cilia · Filtration rate · *Mercenaria mercenaria* · *Mytilus edulis*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

In a recent paper, Specht & Fuchs (2018) claim that 2 ciliary suspension-feeding bivalves, the blue mussel *Mytilus edulis* and the hard clam *Mercenaria mercenaria*, differ in how they respond to viscosity, as well as in their ciliary control mechanisms. For *M. mercenaria*, the authors found no change in filtration rate or beat frequency of water-pumping cilia in response to changes in viscosity at constant temperature. For *M. edulis* on the contrary, Riisgård & Larsen (2007) found these parameters to depend on manipulated viscosity at constant temperature in the same

way as viscosity changes with temperature. This trend of temperature-dependent viscosity of ambient water strongly controlling bio-mechanical activity associated with low Reynolds number flow has been confirmed by many studies ever since that of Podolsky (1994). The surprising and opposite conclusion by Specht & Fuchs (2018) warrants a closer examination of their findings.

Specht & Fuchs (2018) conducted laboratory experiments to quantify the effects of temperature and viscosity on filtration rate (clearance rate, or pumping rate) and on water-pumping ciliary beat frequency in *M. mercenaria*. Thus, the viscosity was adjusted to

*Corresponding author: hur@biology.sdu.dk

known temperature equivalents using PVP (polyvinylpyrrolidone), and ciliary beat frequency was studied on excised gills placed on a microscope cover slip in viscosity-adjusted seawater at room temperature. Clearance rates of intact clams positively correlated with temperature, but unfortunately the effect of temperature on ciliary beat frequency was not measured. However, the effect of PVP-manipulated viscosity at constant temperature showed that filtration and ciliary beat rates were not correlated with viscosity. Based on these observations, Specht & Fuchs (2018) concluded that the increase in filtration rate with temperature was not caused by viscosity effects on ciliary beating, but was probably due to physiological effects of changing temperature. This conclusion is contrary to previous findings for *M. edulis* by Riisgård & Larsen (2007), who showed that viscosity controls the filtration rate of intact mussels as well as the beat frequency of water-pumping lateral cilia studied in gill preparations. Therefore, to reconcile the opposing views, Specht & Fuchs (2018) suggested that the 2 ciliary suspension-feeding bivalves 'fundamentally differ in their responses to viscosity' (p. 137) and that the 2 species 'differ in their ciliary control mechanisms' (p. 129). However, this conclusion is unwarranted.

METHODOLOGICAL ISSUES

Specht & Fuchs (2018) claim that their viscosity results differ from the previous findings by Riisgård & Larsen (2007) for *M. edulis* 'although the methodology was generally similar between the 2 studies' (p. 137). However, the ciliary study conducted by Riisgård & Larsen (2007) was based on the technique developed by Nielsen et al. (1993) and Riisgård et al. (1996), who used one-filament-thick mussel gill preparations elevated 1 cm above the bottom and stretched to obtain the normal interfilament distance in a microscope observation chamber. Isolation of the mussel gill inactivates the lateral cilia (*lc*), whereas the laterofrontal cirri (*lfc*) remain active (Riisgård et al. 2015). The *lc* can be reactivated by means of 10^{-6} M serotonin, and Riisgård & Larsen (2007) studied the beat frequency of *lc* by measuring the metachronic wave length and its speed. Specht & Fuchs (2018) did not add serotonin to their hard clam gill preparations, resulting not only in different methodology but also a serious issue with the interpretation of their results (see next paragraph).

The supplementary video of Specht & Fuchs (2018) reveals that no metachronic waves of water pumping

lc can be seen, only some beating activity of compound *lfc*. From Table 5 in Specht & Fuchs (2018) it appears that the *lc* in *M. edulis* have a width of 0.2 μm (which is the usual diameter of a single cilium), whereas the 'lateral cilia' in *M. mercenaria* apparently have a 4 times larger width (indicating compound *lfc*). Further, Table 5 in Specht & Fuchs (2018) shows that the ciliary beat frequency in *M. edulis* decreases from (1/0.043 =) 23 Hz at 21°C to 13 Hz at 5°C, which is typical for *lc* (Jørgensen & Ockelmann 1991, Nielsen et al. 1993, Riisgård et al. 2015), whereas the beat frequency in *M. mercenaria* was only 8 Hz at 21°C and 7 Hz at 5°C, which is typical for *lfc* (Dral 1967, Riisgård et al. 1996). On this basis, we suggest that Specht & Fuchs (2018) have made a serious mistake by assuming *lfc* to be *lc* in clams. Concurrent observations on mussel-gill preparations would not only have been advantageous for comparison with the previous literature but would presumably have disclosed the misidentification of the *lfc*.

Another shortcoming is that the filtration rate measured at 23.5°C (Specht & Fuchs 2018, their Fig. 1A) is about 5 times higher than in the viscosity-adjusted experiment (their Fig. 1B), where no PVP was added at 23.5°C. A similar lack of agreement between filtration rates measured at 23.5°C (Fig. 2A,B in Specht & Fuchs 2018) undermines the conclusion that clearance rates were not correlated with viscosity; instead, the clams may not have been fully open and filtering at optimal rates in the PVP experiments. To illustrate this point, we note that if the 'control value' of 75.6 $\text{ml h}^{-1} \text{clam}^{-1}$ (from the viscosity experiment at 23.5°C; Table 2 in Specht & Fuchs 2018) were in fact the expected same value of 120 $\text{ml h}^{-1} \text{clam}^{-1}$ (as the filtration rate at 23.5°C from Specht & Fuchs's temperature experiments in their Table 2), then the viscosity data would show an expected viscosity effect at constant temperature, at least for a temperature-tolerance range between 12 and 23.5°C ('because feeding was inhibited below 12°C', Specht & Fuchs 2018, p.136).

The filtration rate of *M. edulis* as a function of acute change in temperature was measured by Kittner & Riisgård (2005) by means of the clearance method in a group of mussels seasonally acclimated to 18°C. This was done by stepwise changes in temperature in order to both determine the temperature-tolerance interval within which the mussels were fully open and to ensure that the acute effects were reversible. The filtration rate as a function of temperature could be expressed by a linear regression line in the temperature-tolerance interval (8 to 20°C). Later, Riisgård & Larsen (2007) measured the filtration rate of

M. edulis without and with different concentrations of PVP added in order to manipulate the viscosity. The relationship between filtration rate of intact mussels at constant temperature (22°C) versus different PVP manipulated viscosities of seawater could also in this case be expressed by a linear regression line, and therefore Riisgård & Larsen (2007) concluded that the ciliary response (beat frequency of *lc* versus viscosity determined for mussel-gill preparations) also applies to intact mussels. Specht & Fuchs (2018, Fig. 1A therein) also found that the filtration rate of *M. mercenaria* correlated linearly with temperature (despite large data scatter), in agreement with similar studies in e.g. the ciliary suspension-feeding soft clam *Mya arenaria* (Riisgård & Seerup 2003); however, Specht & Fuchs suggested that the filtration rate of *M. mercenaria* was not correlated with viscosity and 'likely driven by the physiological effects of changing temperature' (p. 136). This conclusion is unwarranted due to the methodological shortcomings pointed out above.

The effect of temperature on the mussel pump was studied by Jørgensen et al. (1990), who found that filtration rate increased with temperature, linearly correlated with the temperature-dependent decrease in viscosity of water corresponding to the varying viscous resistance to water flow in the canal system of the mussel pump. This general fluid mechanical aspect that applies to all kinds of flow has not been considered by Specht & Fuchs (2018), who exclusively rely on 'physiological effects associated with changes in metabolism or oxygen demand' (p. 136) and hypothesize that hard clams may compensate for less dissolved oxygen in warmer water with higher pumping rates. However, exact and substantiated demonstrations of possible basic physiological parameters involved in the suggested mechanisms of compensatory regulation of the filtration rate in suspension-feeding bivalves seem to be lacking (Riisgård 2001, Riisgård & Larsen 2015, Riisgård et al. 2015, Tang & Riisgård 2016), but there are 2 opposing schools of thought on whether filtration rate is physiologically controlled (Chapter 4 in Gosling 2015).

Many studies have shown that temperature-dependent viscosity of the ambient water controls or strongly affects bio-mechanical activity such as beat frequency of water-pumping cilia in mussels and ascidians, and swimming velocity of sperm cells, ciliates and small aquatic organisms using cilia or small appendages for propulsion (Larsen & Riisgård 2009). Due to a general close matching of the response to power-law regressions for viscosity manipulation (by

means of PVP and other additives) and/or temperature, Larsen & Riisgård (2009) found that viscosity rather than biological mechanisms control the ciliary response. Considering the methodological shortcomings we identified in Fuchs & Specht (2018), the results on hard clams from that study do not affect our present understanding of the effect of temperature on ciliary suspension-feeding bivalves.

LITERATURE CITED

- Dral ADG (1967) The movement of the latero-frontal cilia and mechanism for particle retention in the mussel (*Mytilus edulis* L.). *Neth J Sea Res* 3:391–422
- Gosling E (2015) *Marine bivalve molluscs*. John Wiley & Sons, Chichester
- Jørgensen CB, Larsen PS, Riisgård HU (1990) Effects of temperature on the mussel pump. *Mar Ecol Prog Ser* 64: 89–97
- Jørgensen CB, Ockelmann K (1991) Beat frequency of lateral cilia in intact filter-feeding bivalves: effect of temperature. *Ophelia* 33:67–70
- Kittner C, Riisgård HU (2005) Effect of temperature on filtration rate in the mussel *Mytilus edulis*: no evidence for temperature compensation. *Mar Ecol Prog Ser* 305:147–152
- Larsen PS, Riisgård HU (2009) Viscosity and not biological mechanisms often controls the effects of temperature on ciliary activity and swimming velocity of small aquatic organisms. *J Exp Mar Biol Ecol* 381:67–73
- Nielsen NF, Larsen PS, Riisgård HU, Jørgensen CB (1993) Fluid motion and particle retention in the gill of *Mytilus edulis*: video recordings and numerical modelling. *Mar Biol* 116:61–71
- Podolsky RD (1994) Temperature and water viscosity: physiological versus mechanical effects on suspension feeding. *Science* 265:100–103
- Riisgård HU (2001) Physiological regulation versus autonomous filtration in filter-feeding bivalves: starting points for progress. *Ophelia* 54:193–209
- Riisgård HU, Larsen PS (2007) Viscosity of seawater controls beat frequency of water-pumping cilia and filtration rate of mussels *Mytilus edulis*. *Mar Ecol Prog Ser* 343:141–150
- Riisgård HU, Larsen PS (2015) Physiologically regulated valve-closure makes mussels long-term starvation survivors: test of hypothesis. *J Molluscan Stud* 81:303–307
- Riisgård HU, Seerup DF (2003) Filtration rates in soft clam, *Mya arenaria*: effects of temperature and body size. *Sarsia* 88:415–428
- Riisgård HU, Larsen PS, Nielsen NF (1996) Particle capture in the mussel *Mytilus edulis*: the role of latero-frontal cirri. *Mar Biol* 127:259–266
- Riisgård HU, Funch P, Larsen PS (2015) The mussel filter-pump—present understanding, with a re-examination of gill preparations. *Acta Zool* 96:273–282
- Specht JA, Fuchs HL (2018) Thermal and viscous effects of temperature on *Mercenaria mercenaria* suspension feeding. *Mar Ecol Prog Ser* 589:129–140
- Tang B, Riisgård HU (2016) Physiological regulation of valve-opening degree enables mussels *Mytilus edulis* to overcome starvation periods by reducing the oxygen uptake. *Open J Mar Sci* 6:342–352