



Bivalve molluscs as biosensors of water quality: state of the art and future directions

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Abstract Biological early warning systems (BEWSs) monitor the behaviour or physiology of living organisms as an indirect mechanism to sense local environmental changes, and have become a widely established tool for monitoring water pollution. Complementary to conventional chemical and physical techniques, their strength lies in the ability to continuously monitor water quality, providing direct and rapid warning of toxic discharges. Bivalve molluscs (mussels and clams) are ideal sensing organisms for BEWSs, owing to their: high water filtration capacity; sensitivity to numerous, diverse pollutants; considerable longevity; high abundance; and wide range of physical responses to environmental change. Two behavioural metrics of bivalves have dominated in ecotoxicological studies, and consequently spawned development into BEWSs: valve movements, typically measured using electromagnetic techniques; and bivalve cardiac activity, typically measured using infrared photoplethysmography. However, other, less studied bivalve behaviours may exhibit pollutant impacts, including shell growth and dissolution, premature egg/larval release, horizontal movement, and burial. Such metrics may, with further research,

provide bases for future BEWSs, some through current valve movement monitoring technology, others potentially through acoustic telemetry. Future bivalve-based BEWSs may become more informative and sensitive through the greater nuance that lies in integration of different metrics simultaneously and/or multi-species monitoring. Future directions may seek techniques that reduce disturbance to bivalves and enable observation of a more natural mode of behaviour, through using untethered, or entirely unquipped, specimens. With rigorous controls for individual- and population-level variation, bivalves have great power as biosensors and, bolstered by the scope for future advancements, this field has the potential to make significant contributions to future water quality management.

Keywords Biological early warning system · Biomonitoring · Pollution · Mussel · Valve movement behaviour · Heart rate

Introduction

Aquatic environments frequently experience chemical pollution, the origin of which may be a point source (e.g. governmental or industrial wastewater) or non-point source (e.g. airborne deposition, or runoff from urban or agricultural regions) (Connon et al., 2012). It is essential to protect drinking water sources from such chemical contamination, the discharge of which

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can often be sporadic and unpredictable (White et al., 2002). Situations of accidental or deliberate contamination must be detected rapidly and a speedy response coordinated, owing to the potential for serious impacts on human health (Storey et al., 2011). Consequently, early warning monitoring has been developed, permitting rapid identification of adverse water quality changes.

Most countries employ physical and chemical sensors in the real-time monitoring of aquatic environments. However, physical and chemical sensors are expensive to maintain and reveal only local, short-term water quality changes (Bae & Park, 2014). Moreover, many chemical spill events occur sporadically and contain unpredicted pollutants, and so often go undetected (Mikol et al., 2007). Chemical monitoring of aquatic environments is further hindered by the wide diversity of chemical classes that may be present, ranging from polar and nonpolar organic molecules, to metals and ionic species (Mikol et al., 2007). Additionally, chemical analytical techniques may not capture the toxic effects of chemical mixtures which may be additive, synergistic or antagonistic (Costa et al., 2011, 2012).

The complexity of quantifying the plethora of chemical pollutants continuously, in real-time, including both emerging pollutants and transformation products, combined with the inability to measure toxicity directly, has led to the employment of biomonitors in aquatic environments (White et al., 2002; Cannon et al., 2012). Biological early warning systems (BEWSs) have been built to monitor the responses of organisms, based on behaviour or physiology, allowing continuous sensing of a wide range of pollution (Bae & Park, 2014). Alongside the test organism, BEWSs have two further elements: an automated detection system, and an alarm system (Gerhardt et al., 2006). Hence, altered responses, resulting from pollution, can be rapidly detected and the alarm raised, allowing appropriate action to be taken.

The organisms deployed in BEWSs for aquatic pollution span various trophic levels and range from vertebrates (e.g. fish) to macroinvertebrates (e.g. molluscs, crustaceans, and insects) and microbes (e.g. bacteria and algae) (Gerhardt et al., 2006; Bae & Park, 2014). BEWSs using animals typically employ behavioural parameters, since these are amongst the fastest and most sensitive indicators of pollutants, and their monitoring is usually non-invasive and

relatively inexpensive (Gerhardt et al., 2006; Gerhardt, 2007; Bae & Park, 2014). Bivalve molluscs possess a unique and desirable assortment of characteristics, making them suitable for use in a BEWS, including their high abundance and wide distribution, predominantly sessile nature, ease of handling, and high bioaccumulation ability (Markert et al., 2003). Bivalves have been employed as the sensing organisms in BEWSs, from widespread routine monitoring of surface waters (e.g. Borchering, 2006) to integration within drinking water facilities (McGuire, 2019; Micu, 2020), and extensive research has revealed different metrics by which bivalve behaviour may be assessed to infer stress resulting from contaminants. With increased understanding of bivalve ecology and the emergence of smaller, cheaper and more sensitive technologies, there are opportunities for significant advancements in the development of bivalve-based biosensors. Furthermore, bivalve-based BEWSs may help improve bivalve conservation practices; since the survival of many bivalve species is imperilled by our poor understanding of their ecology, and of anthropogenic influences thereon (Bogan, 1993), bivalve biosensors can improve our knowledge and highlight damaging activities. Additionally, assessment of the health of bivalves in their environment with such BEWSs may allow them to act as sentinels for other organisms (Aldridge et al., 2007; Chowdhury et al., 2016). Lastly, there is also clear potential benefit to the bivalve aquaculture industry; such biosensors can help inform farming decisions, improving animal welfare and also the sustainability and profitability of farming practices (Andrewartha et al., 2015).

Various informative reviews exist on the topic of BEWSs and general on-line biomonitoring approaches (e.g. Gerhardt et al., 2006; Mikol et al., 2007; Bae & Park, 2014). These can provide a useful overview to the area, with explanations of the principles, examples and case studies of ways different organisms have been employed in monitoring, and approaches to processing data for such systems. However, since bivalves do not form the key focus, many of the finer details on the employment of bivalves are vague or missing. This review will primarily focus on the different metrics by which bivalve behaviour has been, or may yet be, evaluated, in response to external factors, chiefly pollutants. Various technologies that have been deployed for their measurement will be explored

and compared, with particular focus on those compatible with field use and automated sensing (key for BEWSs). Relevant case studies will be provided, as well as suggestions of potential areas for further research and development.

Methods to characterise bivalve behaviour

Bivalves largely fall into two groups when being selected for use in biosensors: sessile and mobile species. In their natural habitat, sessile bivalves cement themselves to hard substrates using byssus threads and include the freshwater zebra and quagga mussels (*Dreissena* spp.), the brackish water false mussels (*Mytilopsis* spp.), and the marine mussels, such as *Mytilus* spp. and *Perna* spp. Mobile bivalves are non-cementing and locomote through sediments using an enlarged, muscular foot; they include the freshwater unionid mussels such as *Unio* spp. and *Anodonta* spp., the brackish clams such as *Rangia* spp., and a wide number of marine taxa. A diverse array of metrics exist by which to measure bivalve behaviour, all with potential for development into BEWS assemblies (Fig. 1). Of these, the two best studied are valve movements and cardiac activity, metrics applicable in both sessile and mobile species. This section provides an explanation and evaluation of both metrics, and of the techniques employed to measure them.

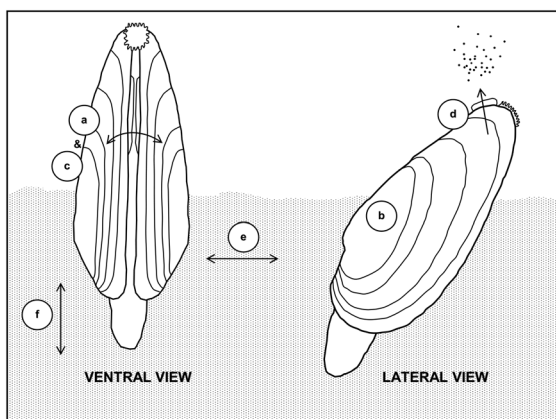


Fig. 1 Metrics of bivalve behaviour: a—valve movements; b—cardiac activity; c—bivalve growth; d—release of eggs and glochidia; e—horizontal movement; f—burial

Valve movements

Background

Valve movements (Fig. 1a) are a well-established metric of bivalve behaviour, which has received considerable attention in the literature. Interest began over 100 years ago (Marceau, 1909), expanding significantly over the past thirty to forty years. Starting with laboratory experimentation and evolving to field-based studies, commercial BEWSs now exist, employing valve movements to indicate unfavourable water conditions. Mussel monitors first appeared on the River Rhine in 1992 and various valve monitors exist worldwide (White et al., 2002); for a review on the principles of valve movement use, set in the context of an established proprietary commercial BEWS, see Kramer & Foekema (2001). Through dose-dependent (e.g. Liu et al., 2016) and time-dependent (e.g. Tran et al., 2003) studies of bivalve responses, sophisticated modelling can allow prediction of changes resulting from contaminants. Systems have even been proposed to quantitatively estimate contaminant concentration from observed valve movement changes (Jou & Liao, 2006; Chen et al., 2012).

Using valve movements to monitor contaminants relies on the bivalve's ability, upon detecting a perturbation (e.g. toxic chemical stressor), to alter its behaviour; this may involve counteracting the change, or else, to lower or isolate as far as possible its exposure to ambient water. Most species maintain open valves during submersion, permitting feeding and respiration. However, prolonged closure (especially coordinated closure among multiple individuals) often indicates an environmental perturbation, due either to natural changes in conditions, or pollutant(s) (Kramer et al., 1989; Kramer & Foekema, 2001; Barile et al., 2016). Responses to pollutants depend on the local physical conditions, sensitivity of the individual concerned, and nature of the stressor itself (toxicity/concentration). Potential responses to toxicants include: closing of valves for a fixed period of time (avoidance behaviour) (e.g. Ait Fdil et al., 2006; Giari et al., 2017); flapping (increasing pumping), i.e. rapid cycles of abduction (valve-opening) and adduction (valve-closing) (e.g. Basti et al., 2009; Bamber & Westerlund, 2016); narrowing valve gape (lowering/ceasing pumping) (Ait Fdil et al., 2006; Giari et al.,

2017); wide gaping, indicative of death (Kramer & Foekema, 2001; Andrade et al., 2016).

In analysing valve movement behaviour, various approaches and endpoints can be considered. Valve position is typically expressed as percentage of maximum opening (e.g. Comeau & Babarro, 2014; Clements et al., 2018), angle in degrees (e.g. Robson et al., 2007; Powell et al., 2017), or in units of distance (across valve gape) (e.g. Liu et al., 2016; Basti et al., 2009). Behaviour is profiled at a defined rate (dependent on technique capabilities or desired frequency) and various statistics extracted, used to assess traits of normal behaviour and effects of perturbations. Typical attributes measured include: time spent in open/closed positions (e.g. Chambon et al., 2007; Hartmann et al., 2016); average valve openness over a set time interval (e.g. García-March et al., 2008; Liu et al., 2016); frequency of valve movements (e.g. Liu et al., 2016; Hartmann et al., 2016); speed of movements (e.g. Robson et al., 2007; Durrieu et al., 2016); distance travelled (reflecting the amount of valve movement) (e.g. Redmond et al., 2017; Bamber & Westerlund, 2016); cases of excessive opening (gaping, suggestive of death) (Kramer & Foekema, 2001; Andrade et al., 2016). Different pollutants, or concentrations thereof, can impact different attributes to varying degrees; therefore, the most sensitive (and informative) varies with pollutant type or concentration (e.g. Liu et al., 2016; Giari et al., 2017; Redmond et al., 2017), rendering analysis of a range of attributes advantageous.

Established BEWSs typically measure and average attributes over set time intervals, e.g. five (Borcherding, 2006) or fifteen minutes (Kramer & Foekema, 2001), comparing with previously collected data. Alternatively (especially for large, high-frequency datasets), behaviour of individuals may be modelled, allowing more sophisticated behavioural analysis; various techniques have proved effective (Jou & Liao, 2006; Schmitt et al., 2011; Sow et al., 2011; Azaïs et al., 2014). Historical data allows a “learning period”, in which baseline thresholds of behaviour (in the monitored environment) are defined, allowing divergence to be identified in newly collected data (Kramer & Foekema, 2001). Comparison may employ whole group averages (e.g. Borcherding, 2006), or separate individual averages (e.g. Kramer et al., 1989; Sow et al., 2011), with the latter often considered more informative (e.g. Redmond et al.,

2017). When newly collected data exceeds thresholds of normal behaviour, alarms can alert of a potential perturbation (e.g. Kramer et al., 1989); triggering typically stems from significant deviation from normal behaviour by a pre-determined proportion of individuals.

Assessment of valve movement attributes may also be used in toxicity testing; attributes are assessed in sets of exposed individuals and compared with controls, allowing measurement of toxicant reference levels for different species (e.g. Curtis et al., 2000; Ait Fdil et al., 2006). Sensitivity naturally depends on the species involved (e.g. Liu et al., 2016), whilst a range of factors (that define the conditions of the exposure experiments) can affect the recorded toxicity, including: physical conditions like temperature (Borcherding, 2006); types of attribute examined (e.g. Redmond et al., 2017); number of specimens tested (e.g. Borcherding, 1992); and length of exposure time (e.g. Liao et al., 2009). Reference levels indicate animal sensitivity and, thereby, have utility in BEWS coordination. However, owing to their specificity, they are of limited applicability beyond the controlled settings they were generated under; the natural environment (where BEWSs typically operate) contains diverse mixtures whose compound effects can vary markedly from those of individual contaminants (Costa et al., 2011, 2012).

Advantages and disadvantages of measuring valve movements

Certain stressors can induce specific valve movement patterns, potentially enabling pollutant identity to be inferred. For example, mercury induces a distinctly different pattern of behaviour to other trace metals in the Asian Clam, *Corbicula fluminea* (O.F. Müller, 1774) (Tran et al., 2007). However, quantitative estimation, or identification of pollutants, is beyond the scope of most valve movement monitors in most settings. Consequently, BEWSs developed using this metric benefit from deployment with chemical and physical monitoring strategies. For instance, a sudden alteration in valve movements of zebra mussels [*Dreissena polymorpha* (Pallas, 1771)] was detected using a commercial BEWS on the River Ruhr at Fröndenberg (North Rhine-Westphalia, Germany) in 1994, triggering an alarm; a water sample was taken, later analysis of which revealed a chemical pollutant which

acted as a ‘fingerprint’, enabling identification of the company responsible for its discharge (Borcherding, 2006).

Despite their utility, valve movements exhibit responses to various factors that may be independent of exposure to pollutants, including changes to respiration, feeding, metabolic activity, excretion, locomotion, and reproductive activity (Nagai et al., 2006; Robson et al., 2009; Coquereau et al., 2016; Clements et al., 2018). Therefore, valve movements can be highly variable within an individual. Furthermore, owing to natural variation of such factors between separate individuals, and (more so) in separate species, valve movements diverge between individuals (Kramer & Foekema, 2001) and species (e.g. Kramer et al., 1989; Liu et al., 2016). Valve movements also alter under various ambient factors like temperature, season, food availability, and physical vibrations (Kramer & Foekema, 2001; Borcherding, 2006; Clements et al., 2018). Furthermore, valve movements exhibit rhythms (i.e. coordinated patterns of opening/closing), regulated by external physical factors like light (e.g. Garcia-March et al., 2016; Comeau et al., 2018). For instance, circadian rhythms exist in raft-cultivated mussels (*Mytilus galloprovincialis* Lamarck, 1819) in Spain, where maximum valve opening occurs at night, and minimum by day (Comeau et al., 2018). Rhythms can be highly uniform. To minimise their potential to trigger alarms falsely, rhythms should be: accounted for, e.g. by modelling rhythmic patterns to allow dynamic changes in baseline reference data (e.g. Sow et al., 2011); or avoided, by control of the physical factors governing them.

However variation within and between individuals is accounted for, rigorous testing of behaviours under a variety of conditions is key for BEWS development. Laboratory environments allow strict control of ambient conditions under which pollutant effects may be gauged, but can affect natural rhythms, impairing understanding of typical behaviour (Wilson et al., 2005; Garcia-March et al., 2016); further, thorough characterisation in the field is therefore advisable. Measurement of physical conditions, e.g. temperature, pH, light levels, or turbidity, is often necessary in BEWSs, to allow dynamic changes to thresholds, improving alarm accuracy (e.g. Borcherding, 2006). Furthermore, isolation of subjects from predators is wise, not only to protect cohorts and their attached

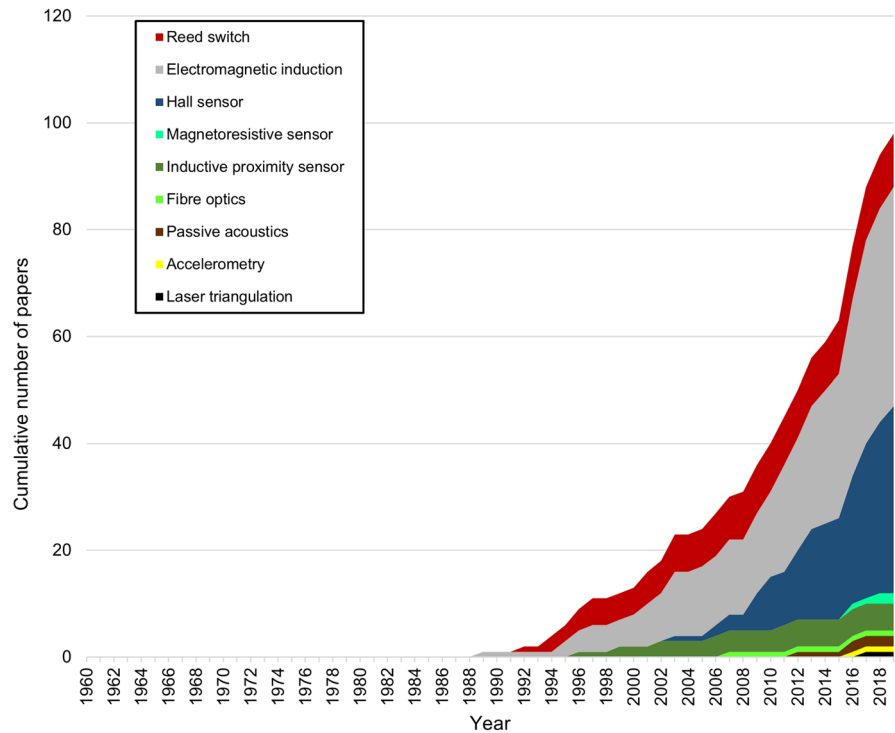
equipment, which predators may dislodge (e.g. García-March et al., 2008), but also because predators may impact behaviour; bivalves are sensitive to cues of predators, such as damage to conspecifics, which causes individuals to alter valve gape behaviour so as to lower their chances of detection (Robson et al., 2007; Dzierżyńska-Białończyk et al., 2019). One last consideration is the fact that, once bivalves close upon pollutant exposure, they can remain closed long after favourable conditions return, limiting their utility in the interim (Kramer & Foekema, 2001).

Technologies

A raft of different techniques exist for measuring valve movements. Some are mechanical in nature, typically inferring or measuring physical displacement of a thread or wire attached to the free valve of a fixed specimen (e.g. Englund et al., 1994; Markich et al., 2000). However, mechanical techniques have largely fallen out of use, being less robust and more cumbersome than more recent approaches, and better suited to laboratory use. Alternatively, video and image analysis techniques may be employed; videos or photographs of bivalves may be analysed by eye or using image analysis software, often using a known visual cue (e.g. a shell tag for tracking, or an adjacent reference object of known dimensions) to gauge openness of valves (Newell et al. 2001; Sénéchal et al. 2008; Speiser & Johnsen, 2008; Coquereau et al., 2016; Dzierżyńska-Białończyk et al., 2019). Video and image analysis techniques also typically lack field compatibility, as well as the necessary automated detection of typical BEWSs. However, numerous alternative approaches exist (Fig. 2), capable of remote, automated valve movement detection. Many have proved appropriate for field use, and certain techniques have been developed into BEWSs.

Reed switch Reed switches are simple electrical switches that respond to magnetic fields. These devices have been applied to valve movement monitoring, principally in a well-established commercial BEWS, the Dreissena-Monitor (Envicontrol, Germany); this system employs 84 zebra mussels, *Dreissena polymorpha*, divided equally between two channels (Borcherding, 2006), fixed onto supports, and equipped with a magnet on the free valve. With a reed switch positioned opposite free valves, opening (or closing) of

Fig. 2 Cumulative number of published papers using valve movement sensing technology, compatible with BEWS use, between 1960 and 2019. Note the order in the legend reflects the order of the plots. Data were collated using a systematic review from a Web of Science literature search, on 22/11/19, with advanced search term: TS=(bivalve* OR mussel* OR clam* OR scallop* OR oyster*) AND TS=(sensor* OR biosensor* OR sense* OR sensing OR detect* OR detector* OR detection OR activity OR movement*) AND TS=(valve* OR gape* OR gaping). Each publication was read to identify whether one or more technologies were used



valves acts to close (or open) the reed switch, allowing a simple binary readout (open/closed) for all mussels. This is robust, reliable and effective for water quality monitoring (e.g. Borcherdig, 2006). Magnetic relay sensors may be used analogously to provide binary information (Irlandi & Mehlich, 1996). However, binary measurement conceals intricacies of valve movement which most modern techniques measure as a continuous variable; even graded bands of openness, gauged using multiple reed switches (García-March et al., 2008), may obscure behavioural nuances. A computer records average percentages of open mussels and number of valve movements for the Dreissena-monitor. Using such large cohorts helps nullify individual peculiarities, and is a particular strength of this technique. Furthermore, although most alternative electromagnetic induction techniques require a continuous draw of electricity (García-March et al., 2008), reed switch energy consumption is low, making it ideal for more remote BEWS deployments, such as general surface water monitoring, where mains power (power grid electricity) may be unavailable.

Dreissena-monitor alarms are set, based upon dynamic limits, which are informed by toxicity testing. This may be carried out using the same device.

Instead of feeding live water through both channels, with each acting as validation for the other (during continuous water monitoring), a toxicant may be applied to one (test) channel, the other channel acting as a control; differences in opening/closing behaviour between the channels helps determine toxicant reference levels (Borcherdig, 2006).

Electromagnetic induction Electromagnetic induction forms the key principle of many valve movement techniques (Véró & Salánki, 1969; Kádár et al., 2001; White et al., 2002), including another commercial BEWS, the Musselmonitor® (Delta Consult B.V., The Netherlands), and the high-frequency non-invasive (HFNI) valvometry technique (“Molluscan Eye”, n.d.). Systems employ two small coils, either one on a support the bivalve is fixed to and the other on the free valve (for the Musselmonitor®), or one to each valve of freely moving specimens (in the HFNI set-up). A high-frequency oscillating current is passed through one coil (transmitter), generating a magnetic field, which induces a current in the second coil (receiver) inversely proportional to the distance between coils (Kramer et al., 1989; Curtis et al., 2000; Chambon et al., 2007; Barile et al., 2016; Giari et al., 2017). The

range of operation is set such that the generated signal is (essentially) linearly related to the distance.

The Musselmonitor® can be deployed either as an in situ or flow-through system, with eight mussels. A learning period informs later evaluation, performed on behaviours of individuals. Such evaluation and measurement settings can be optimised for the conditions of use, allowing definition of sensible threshold criteria and appropriate alarm generation (Kramer & Foekema, 2001; Giari et al., 2017). This system is effective, the measurements robust, and the statistics performed simple (involving averages of attributes within individuals over set time intervals). However, this simplicity may average out some behavioural intricacies. The device must connect directly to a PC to allow data visualisation, although remote data-logging is possible through a networking module. Furthermore, the in situ version can operate in the field without mains power, using an internal, rechargeable battery (Kramer & Foekema, 2001). Therefore, the Musselmonitor® is suitable for a broad range of BEWS settings, from remote deployments of the in situ version, to controlled deployments of the flow-through version, e.g. in drinking water facility monitoring, with connected computer(s).

The HFNI valvometry system, employing 16 bivalves, possesses two electronic cards: The first sits in the water and measures valve activity; the second remains outside the water, drives the first card, and manages the transfer of the data to a central workstation, where it is processed (Schwartzmann et al., 2011; Sow et al., 2011). This system seeks to enable free movement, with flexible wires and no fixing; this is advantageous, as fixing may alter valve movement patterns (Kramer & Foekema, 2001), and is generally most suitable for sessile, byssus-forming bivalves. The HFNI technique is therefore suitable for application with both sessile and mobile species. Although each valve is equipped, sensors are lightweight, and appear to effect no difference on valve movement behaviours (Tran et al., 2003), though this naturally depends on the size and species of bivalve used. The HFNI system has many further advantages, including its ability to employ low power consumption in field-based units and broadcast data to a central workstation for processing. Furthermore, the sensors have in-built anti-fouling and are maintenance-free (Andrade et al., 2016). Data are collected at a high frequency and modelled, potentially allowing discernment of

intricacies that slower data collection rates and simpler data processing protocols may overlook. The HFNI system is an effective technology for BEWS monitoring, having been widely demonstrated in marine monitoring. The principles of the system are robust and, since remote use is not a problem, such a system could be analogously applied in various other BEWS settings, from monitoring of surface waters to drinking water facilities.

Hall sensor Hall sensors have been widely employed in laboratory and field settings, and may form effective BEWSs. The Hall effect sensor is a device whose output voltage alters, depending on the external magnetic field. Through attachment of a Hall sensor to one valve, and a small rare earth magnet to the opposite valve (as is typical), the distance between valves can be measured continuously (Wilson et al., 2005; Nagai et al., 2006; Robson et al., 2007, 2009; Andrewartha et al., 2015; Hartmann et al., 2016; Liu et al., 2016; Clements et al., 2018; Comeau et al., 2018). Magneto-resistive sensors also act as transducers of magnetic field strength, and can be used analogously (Garcia-March et al., 2016).

Hall sensor approaches have many strengths: employing off-the-shelf components, minimal loads are typically applied to valves (lighter than Musselmonitor® coils, marginally heavier than HFNI coils), high voltages (typical in coil-based methods) are not required, and power consumption can be low (Wilson et al., 2005; Nagai et al., 2006; Comeau et al., 2018). Since valves are not usually fixed, the method is suitable for sessile and mobile bivalve species. Hall sensors also permit very high sampling rates; this may be important, since too low a sampling rate can underestimate adduction and abduction events, causing potential information loss or biases in results (Robson et al., 2009). The Musselmonitor® and HFNI systems (which employ sequential data collection to prevent interference of separate coil pairs) may exhibit such information loss through slower sampling rates. However, though not routinely a problem, Hall sensor systems may experience interference if multiple equipped animals are kept in close proximity. Furthermore, Hall sensor output is not linearly related to the distance, so rigorous and repeated calibration is recommended (Wilson et al., 2005). Overall, Hall sensors provide a promising

BEWS technique, and should be easily adaptable into real-world BEWS applications in a broad range of settings.

Optical techniques Attachment of a light-emitting fibre and light-receiving (and measuring) fibre, one to each valve, allows valve openness to be measured through received light intensity (Frank et al., 2007). Alternatively, by focussing a laser beam on the valve edge of a specimen and measuring the reflected light to gauge distance (a process called laser triangulation), valve movements may also be recorded through received light intensity (Redmond et al., 2017).

Both techniques have been considered for field usage. The fibre optic technique allows isolation of the sensor from the electronics, eliminating any potential for electronic noise (possible with electromagnetic techniques). Fixing is key for both approaches, although laser triangulation may be less intrusive, since no components are fitted onto valves. However, optical techniques may lack the robustness of electromagnetic approaches, since they require good visibility, which is frequently lacking in turbid systems, or where bivalves naturally burrow (Wilson et al., 2005; Robson et al., 2009). Remote deployments, such as general surface water or offshore monitoring, may thereby be incompatible. However, more controlled BEWS settings, such as drinking water monitoring facilities, may be compatible, where: lighting may be artificially provided, when necessary; water to be monitored may have undergone filtration to remove sediment; and mains power provision would negate energy consumption (of sensors/associated computing) concerns. As such, optical techniques do not yet offer a credible technology for ubiquitous BEWS deployment.

Alternative technologies Various alternative approaches may be appropriate in BEWSs, though utility is speculative due to lack of widespread use or immaturity of technology. For instance, inductive proximity sensors have allowed laboratory measurement of valve movements. Generating an electromagnetic field using a high frequency oscillator, metal objects entering the sensor's field cause distortion, altering the amplitude of the oscillator and changing the output voltage (Allen et al., 1996; Miller et al., 1999). By fitting a metal disc to one valve of a fixed specimen, and placing a proximity sensor opposite,

distance changes caused by valve movements are read in voltage changes (Allen et al., 1996; Miller et al., 1999; Ayad et al., 2011). Accelerometers may also allow monitoring of valve movements; as instruments capable of measuring acceleration in three axes, attachment of an accelerometer to one valve of a fixed specimen can allow valve displacements to be calculated (Coquereau et al., 2016). Lastly, experiments with passive acoustics, whereby hydrophones measure sounds produced by different types of valve movements (such as adduction or swimming), has indicated that such a method may allow identification of valve movements in multiple, unattached animals (Di Iorio et al., 2012; Coquereau et al., 2016).

Cardiac activity

Background

Cardiac activity (Fig. 1b) has been widely studied in a range of different bivalves, under laboratory and field conditions, and under various abiotic and biotic factors. It provides information on health, stress, and physiology; since this is impacted by environmental conditions, cardiac activity may be employed as an indicator of alterations in ambient factors (Sarà & de Pirro, 2011; Xing et al., 2016, 2019; Bakhmet et al., 2019). As an integrated organismal response to contamination, it is an effective metric for water quality biomonitoring in bivalves (Kholodkevich et al., 2017). However, baseline cardiac activity is naturally influenced by factors such as respiration (e.g. Gurr et al., 2018) or feeding (e.g. Widdows, 1973), as well as activity like adduction (e.g. Brand, 1972); consequently these must be considered, although cardiac activity can remain constant under large fluctuations in such factors (Thompson & Bayne, 1972).

Employing cardiac activity for monitoring pollutants relies on the bivalve's ability to sense and respond to a perturbation in the ambient environment, such as a toxic contaminant. The response may involve increasing metabolic activity, to counteract the change, or to lower activity and avoid exposure. From cardiac traces, changes are often apparent in the heart rate; heart rate can increase or decrease in response to changes in local water conditions, depending on the pollutant (toxicity/concentration), and the animal's sensitivity. Alterations in rates may sometimes be attributed to direct action of chemical

contaminants on the bivalve. For instance, copper may directly impact nervous stimulation of the myocytes themselves (Nicholson, 2003). However, increases in rate are generally seen as the bivalve heart compensating for stressful situations, increasing their metabolism for excreting toxicants, whilst decreases are often linked to valve closure (a typical isolation response) to avoid stress (Nicholson, 1999b; Sarà & de Pirro, 2011). Indeed, valve closure and heart rate are closely linked; lowering of heart rate usually occurs during valve closure (e.g. Trueman & Lowe, 1971), and cessation of the heartbeat (cardiac arrest) is common, often forming a part of normal, periodic behaviour (e.g. Bakhmet et al., 2005). Upon reopening, heart rate typically increases immediately beforehand, temporarily overshooting baseline rate before restoration of normal rate, likely to allow removal of accumulated anaerobic respiration products (generated during oxygen deprivation associated with valve closure) (e.g. Trueman & Lowe, 1971). Heartbeats can also adopt different patterns in response to pollutants, such as “burst activity” (consisting of series of beats separated by pauses), or entirely irregular patterns (Grace & Gainey, 1987). Such patterns are discernible through closer analysis of the inter-pulse duration. Strength of contractions is given by the amplitude on cardiac traces, whilst the rate-amplitude product may help gauge oxygen consumption. The former is greatest under optimal growth conditions (and decreased by stressors), whilst the latter can inform of stress to the heart (increasing when bivalves counteract stressors through increasing metabolic activity) (e.g. Xing et al., 2019).

In assessing cardiac behaviour, various attributes may be extracted and analysed, ideal as BEWS foundations: heart rate monitoring is typical, as means at set intervals; monitoring of the inter-pulse duration is beneficial, since further information lies in the patterns of heartbeats (Bakhmet et al., 2009) and mean heart rates can hide such intricacies of behavioural response (Depledge et al., 1996); analysis of the amplitude and rate-amplitude product may provide a further insight into pollutant impacts on cardiac activity. Analogously to valve movements, cardiac activity attributes can also be assessed in pollutant exposures to generate toxicant reference levels for different species (e.g. Davenport & Manley, 1978). Measured toxicity naturally depends on specific experimental parameters, like time (e.g. Grace & Gainey, 1987) or

attribute types (e.g. Curtis et al., 2000); its utility is therefore limited, though it may guide deployment or interpretation of BEWSs. Very little has been written on developing bivalve cardiac activity into real-world BEWSs. However, its integration within aquaculture monitoring (Andrewartha et al., 2015) and a commercial BEWS, the Biota Guard marine monitoring system (Biota Guard AS, Norway) (Dragsund et al., 2013), indicates there is value in measuring cardiac activity in BEWSs; small short- or long-term behavioural alterations can inform of local environmental perturbations (Dragsund et al., 2013).

Advantages and disadvantages of measuring cardiac activity

Cardiac activity is a culmination of various physiological factors which respond to perturbations (e.g. chemical stressors). Dose- or time-dependent responses of heart rate to different pollutants can be studied (e.g. Curtis et al., 2000). For instance, copper is an essential trace element for bivalves, though toxic in excess. Using the blue mussel (*Mytilus edulis* Linnaeus, 1758) as a paradigm of bivalve behaviours, copper typically induces bradycardia (reduced heart rate), as well as valve closure and greater heart rate variability (Davenport, 1977; Davenport & Manley, 1978; Grace & Gainey, 1987; Curtis et al., 2000). However, others have reported tachycardia (increased heart rate) as a response (Brown et al., 2004; Bakhmet et al., 2012). These reported differences may be attributed to the varying concentrations of copper applied. Higher concentrations may cause immediate toxic effects, leading to isolation (valve closure) and bradycardia. Lower concentrations may lead to compensatory measures, inducing tachycardia.

Although closely linked, cardiac patterns and valve activity may not always respond simultaneously. Heart rate alone may alter upon chemical application, unaccompanied by altered valve gape (Nicholson, 2003). Therefore, heart rate changes may not always be attributable to valve position (Grace & Gainey, 1987; Curtis et al., 2000), and this metric can reveal independent information on bivalve responses. Furthermore, cardiac activity reveals specimen health through their adaptive capability, since changes in cardiac activity provide an integrated measure of physiological fitness (Turja et al., 2014; Kholodkevich et al., 2017). For instance, when groups of

blue mussels (*Mytilus trossulus* Gould, 1850) from an unpolluted site underwent temporary transplantation to separate sites, either polluted or unpolluted, subsequent toxicity testing revealed differences; those temporarily exposed to polluted sites took longer to recover typical heart rates than those exposed to the unpolluted sites, owing to their impacted health (e.g. Turja et al., 2014).

Unlike many physiological effects, cardiac activity monitoring is simple and robust, easily applied in the field for remote, real-time monitoring of environmental water quality. As the bivalve heart has no endothelial lining, and is directly immersed in the haemolymph, cardiac activity is rapidly affected by toxic substances, enabling rapid detection (Nicholson, 1999a). Furthermore, although heart rate alone may seem a basic index, informative heartbeat patterns can occur in cardiac traces, revealed through simultaneous monitoring of inter-pulse duration. Most techniques for measuring cardiac activity are affected by valve and organ (e.g. foot) movements, causing artefacts in traces, e.g. peaks, slow waves, or baseline drift. These can make heart rate difficult to determine and often renders amplitude unusable (Seo et al., 2016). Although amplitude (or rate-amplitude product) is occasionally used to measure cardiac activity (e.g. Bakhmet & Zdorovenov, 2010; Xing et al., 2019), it is typical that only qualitative information about the strength of contractions (from amplitude) is available (Trueman et al., 1973). Furthermore, cardiac activity traces do not always exhibit the same pattern; single or double (or occasionally more) peaks can occur in the waveforms, making elucidation of cardiac attributes more complex (e.g. Seo et al., 2016).

The high variability in cardiac activity presents additional challenges for monitoring, with variation occurring not only between different species of bivalve (e.g. Marshall & McQuaid, 1993; Bakhmet & Khalaman, 2006; Xing et al., 2016) but also within a species (e.g. Bakhmet et al., 2005; da Cândido & Romero, 2006; Sarà & de Pirro, 2011). Heart rate can regularly decrease to a temporary arrest, without coordination between animals (Bakhmet & Khalaman, 2006), and cardiac activity can alter, depending on the size and weight (Nicholson, 2002; Xing et al., 2016, 2019) or age of individuals (Xing et al., 2016, 2019), and under physiological stresses like spawning (Xing et al., 2016). Furthermore, rhythms (coordinated cardiac behavioural patterns) exist in

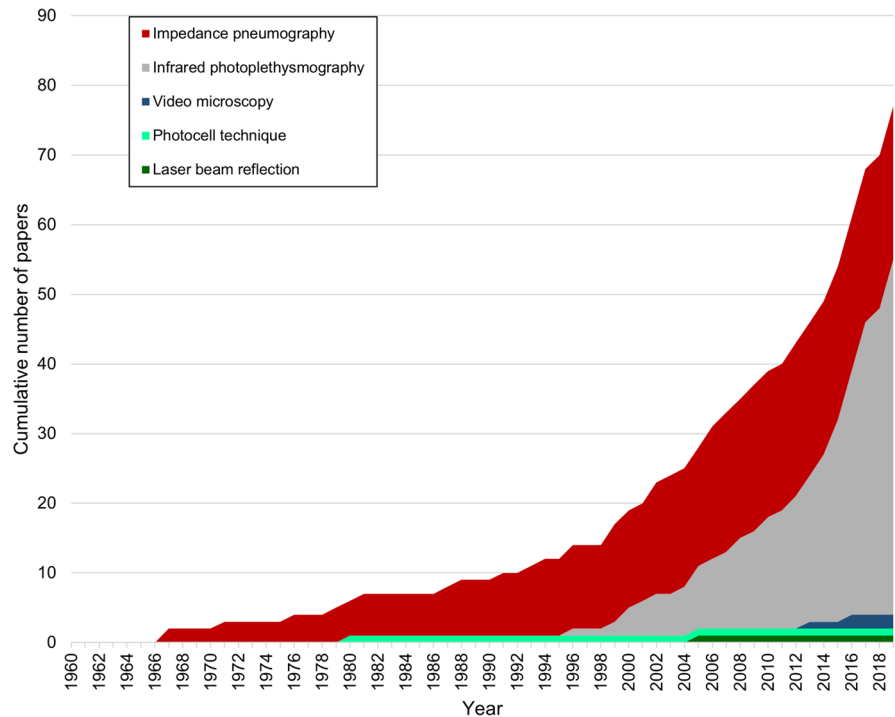
many species, some set by ambient physical factors such as light or tide, others controlled by endogenous biochemical or physiological factors, including reproduction (e.g. Bakhmet & Khalaman, 2006; Bakhmet et al., 2019; Xing et al., 2019). Indeed, heart rate is altered by various physical factors, such as temperature and salinity (e.g. DeFur & Mangum, 1979; Braby & Somero, 2006; Xing et al., 2016). However, rhythms can be altered by laboratory experimentation (Bakhmet & Zdorovenov, 2010) and not all individuals may exhibit the same pattern. Therefore, extensive characterisation of behaviour is key before BEWS employment can be considered, under both laboratory, and field conditions. Encapsulation of individuals within BEWSs is also propitious, not only to protect cohorts (and monitoring equipment) from predator attacks, but also because their presence can impact cardiac activity; the scent of a predator can elevate bivalve heart rate (Rovero et al., 1999).

Bivalve cardiac activity provides a good basis for BEWSs, as exemplified by the commercial system, the Biota Guard marine monitoring system (Drag-sund et al., 2013). After thorough characterisation of behaviours, a suitable monitoring frequency should be established and individual ranges of typical behaviour should emerge, deviations from which should allow sensible alarm triggering. Physical monitoring alongside such a BEWS would be advantageous; since various physical factors impact behaviour, appropriate dynamic adjustment of alarm thresholds should improve sensitivity. By employing an appropriate sample size, sudden alterations (concurrent between multiple individuals) could alert of environmental perturbations.

Technologies

Various technologies have been implemented for measurement of bivalve cardiac activity. The earliest methods were incompatible with BEWS requirements, involving visual inspection of the heart (or proxy of cardiac activity) after shell dissection, to manually count beats (Segal, 1956; Pickens, 1965; Scott & Major, 1972). Visual observation after dissection, or direct microscopic examination (through thin spat shells) remain viable laboratory techniques (Pandolfo et al., 2009; Parker et al., 2015; Liu & Wang, 2016). Similarly, detailed cardiac imaging

Fig. 3 Cumulative number of published papers using cardiac activity sensing technology, compatible with BEWS use, between 1960 and 2019. Note the order in the legend reflects the order of the plots. Data were collated using a systematic review from a Web of Science literature search, on 22/11/19, with advanced search term: TS=(bivalve* OR mussel* OR clam* OR scallop* OR oyster*) AND TS=(heart* OR cardiac) AND TS=(rate* OR activity). Each publication was read to identify whether one or more technologies were used



through ultrasound (Haefner et al., 1996) or magnetic resonance imaging (MRI) (Lannig et al., 2008; Seo et al., 2016; Sukhotin et al., 2017), or measurement of haemolymph flow through Laser Doppler Perfusion Monitoring (Lannig et al., 2008), provide further means of extracting cardiac attributes; but, as expensive, laboratory techniques, these are unsuitable for BEWS constructs. Fortunately, established techniques exist for non-destructive and field-compatible measurement of cardiac activity (Fig. 3).

Impedance pneumography Impedance pneumography was, for a long time, the preferred method. As a technique allowing measurement of pulsatile changes in volume, it is ideal for measuring the contractions of cardiac activity. Useful in the laboratory, and even long-term (at least several weeks) field deployments (Trueman et al., 1973), implementation often involves the drilling of two holes through the bivalve shell (one per valve), each side of the pericardial cavity. Electrodes are inserted, connected by flexible wires to an impedance pneumograph; through applying an oscillating current between electrodes, the latter converts changes in impedance (from varying heart volume) to a voltage signal, often recorded on a pen recorder (Trueman, 1967; Trueman & Lowe, 1971; Trueman et al.,

1973; DeFur & Mangum, 1979; Stickle & Sabourin, 1979; Grace & Gainey, 1987; Marshall & McQuaid, 1993; Stenton-Dozey & Brown, 1994; Nicholson, 1999a, 1999b, 2002, 2003; Braby & Somero, 2006; da Cândido & Romero, 2006).

Flexible wires allow uninhibited movement, and the equipment is comparatively lightweight, weighing no more than supposedly non-impacting infrared photoplethysmographic technology (Kholodkevich et al., 2017); this may lower impacts on behaviour, though it naturally depends on the size/species concerned. However, the approach is particularly invasive; drilling increases stress in specimens, causing higher mortality (under pollutant exposure) (Stenton-Dozey & Brown, 1994), or lower growth (Trueman & Lowe, 1971), than unequipped specimens, as well as potential artefacts (Bakhmet et al., 2009). Since alternative, less invasive approaches exist, it is unlikely to be used more widely for BEWS strategies.

Infrared photoplethysmography Evolving more recently than impedance pneumography, infrared photoplethysmography has become the current preferred technique. Used both in the laboratory and long-term (several months) field deployments (Kholodkevich et al., 2017), commercial BEWSs also employ this

technique. The approach typically involves attachment of a reflective optocoupler onto a bivalve shell over the cardiac region, containing an infrared light-emitting diode and a phototransistor, aligned parallel. The former emits infrared light; reflecting off the cardiac region, received light intensity is then measured (as current) by the phototransistor. Periodic changes of the heart shape and size, associated with cardiac activity, impart a pattern on this light, allowing extraction of cardiac attributes. The unit connects by flexible wires to other components, typically amplifying and filtering the current, and feeding this to an oscilloscope or computer (Depledge & Anderson, 1990; Depledge et al., 1996; Fedotov et al., 2000; Bakhmet et al., 2005, 2009, 2012, 2019; Bakhmet & Khalaman, 2006; Bakhmet & Zdorovenov, 2010; Sarà & de Pirro, 2011; Dragsund et al., 2013; Turja et al., 2014; Andrewartha et al., 2015; Seo et al., 2016; Xing et al., 2016, 2019; Kholodkevich et al., 2017; Zhang et al., 2019). Two optical fibres can alternatively be attached to shells, and used to emit and collect the infrared light of an analogous emitter/receiver unit, allowing it to remain detached from specimens (Fedotov et al., 2000; Turja et al., 2014; Kholodkevich et al., 2017).

Infrared photoplethysmographic approaches generally allow unimpeded movement, with flexible wires. The technique is reputed to be non-invasive (Seo et al., 2016; Xing et al., 2016), with the heavier fibre optic set-up demonstrated not to impact typical behaviours (Kholodkevich et al., 2017), though this would depend on size/species. However, manual shell thinning or drilling can be necessary for thick-shelled specimens (e.g. Andrewartha et al., 2015), necessitating some degree of invasiveness. Nevertheless, power use can be low and the technique is robust, rapid, efficient, and economical (Seo et al., 2016; Xing et al., 2016). The ease of this technique, combined with its lower invasiveness, has likely made it the preferred method, and fostered its integration in real-world BEWS monitoring (Dragsund et al., 2013; Andrewartha et al., 2015). This technique offers the best means for ubiquitous cardiac activity bivalve-based BEWS applications.

Alternative techniques Edge-detection software can allow heart rate to be detected in videos of juveniles, by comparing pixel colour at a reference point (stationary during the cardiac cycle) with that at a point of interest (moving during the cardiac cycle) (Domnik

et al., 2016). This allows non-invasive remote detection of heart rate. However, video microscopy relies on ideal positioning, transparency of shells (atypical beyond juveniles) and good visibility of specimens. Therefore, the technique is unlikely to enter widespread BEWS use. Further optical means may also be exploited to measure cardiac activity, allowing light to be imprinted with rhythmic changes associated with the cardiac cycle, and subsequently measured to extract cardiac attributes. For instance, a lamp may be mounted to one valve, and a photocell (a device whose output voltage alters with light intensity) to the opposite (Dietz & Tomkins, 1980). Compatible with ambient light, and exploiting off-the-shelf components, this technique may have promise, though viability in a field setting (and BEWS potential) is unproved. Alternatively, valves can remain unequipped; a laser beam can instead be directed at the cardiac region (by low-power laser diode), with reflected light collected by a fibre optic bundle, and measured by photodiode (Ritto et al., 2003, 2005). Enabling good resolution, the technique is simple and employs off-the-shelf components. However, precise positioning of specimens is important and the surrounding medium naturally affects readings, so this technique is likely to be of use only in more controlled BEWS settings.

Alternative metrics and future directions

Beyond valve movements and cardiac activity, alternative behavioural metrics (Fig. 1c–f) have received comparatively little attention, particularly in BEWS development. Moreover, monitoring a combination of behaviours (and attributes within them), or a combination of species simultaneously, may generate more nuanced information, producing more informative and sensitive systems. These areas for potential improvement of systems will be explored in this section. Emerging technologies, offering potential improvements and step-changes to both existing and alternative measures of behaviour will also be highlighted. Furthermore, few monitoring designs properly account for variation in pollutant responses within a species, potentially causing faulty adjustment of system sensitivity to levels that are too high (causing false alarms) or too low (causing missed pollution discharges). Suggestions will be provided of how to

tackle this difficulty, to improve current bivalve-based BEWS approaches.

Bivalve growth

Growth of bivalves (Fig. 1c) is impacted by their health and metabolism, altering under: stress, such as from handling (Trueman & Lowe, 1971; Andrewartha et al., 2015); ambient physical factors, like temperature (Zhang et al., 2019); or exposure to chemical stressors (Manly & George, 1977; Muncaster et al., 1990; Bonel et al., 2013; Nobles & Zhang, 2015; Mincy & McKinney, 2019). Changes in growth are often studied manually through weight or length measurements, as well as other forms of physical or laboratory examination, such as sclerochronology (Steinhardt et al., 2016), involving investigation of shell growth rings (Lundquist et al., 2019), or scope for growth (e.g. Zhang et al., 2019), involving measurement of the energy available for growth through various physiological responses. For remote field monitoring, whole animal wet weight is an unsuitable index of growth, owing to the impracticality of remote continuous weight measurement, and the potential uncertainty of measurements, stemming from the varying quantities of water bivalves may hold within their mantles. Therefore, growth is more easily gauged through the shell. Shell growth may possibly be observed using the current valve movement monitoring technology at low frequency (Wilson et al., 2005); the technique measures the distance between the two valves, which should increase as a bivalve grows, with growth originating in the mantle cavity. By this means, (daily) incremental increase in minimum (or maximum) opening distance may be observed as the growth rate (Schwartzmann et al., 2011). Some work has commenced in this area, although greater utility may be realised with further application.

Growth rate changes are slow changes; this metric would not allow very rapid pollution detection, but may be useful for more general water quality monitoring during long-term environmental change, at the scale of months or years. Lowest growth will likely be seen under suboptimal water quality. However, it should be noted that growth may not always lower with increases in contaminants; certain contaminants, e.g. lead and cadmium, positively correlate with

growth in the freshwater mussel *Anodonta anatina* (Linnaeus, 1758) (Manly & George, 1977), and metals can affect the chemical composition of shell material, causing deformation and blistering (Spann et al., 2010). Furthermore, growth can be preferentially directed, sometimes in favour of the soft tissue rather than the shell (Gagné et al., 2001), such that effects may not always be observable, and slow-growing species may offer little information in the limited windows over which specimens may be sensibly applied in BEWSs (Newton et al., 2001). However, growth measurements should generally reflect pollutant impacts.

Growth measurement may be especially useful in recording toxin-related shell dissolution events. Valve closure (in response to a contaminant) is a typical avoidance behaviour, and the resulting limited oxygen availability leads to anaerobic respiration, which results in the formation of acidic species. These are neutralised through the dissolution of calcium carbonate from shells, leading to shell thinning. This effect has been observed in response to various contaminants, as well as unfavourable physical conditions (Crenshaw & Neff, 1969; Akberali et al., 1977; Akberali & Black, 1980; Alyakrinskaya, 2005; Nagarajan et al., 2006). Dissolution occurs on the inner shell surface, and seems to occur primarily from newly deposited calcium, with the least soluble components (e.g. nacreous layer) least likely to experience dissolution (Akberali, 1980; Alyakrinskaya, 2005). Although dissolution is likely more pronounced further from shell edges, it is possible that erosion may also operate at shell perimeters (since these may also act as a buffer for acidic species in the extrapallial fluid), leading to reduced separation of valves (and closer valve sensors). Whether thinning brings valves closer or not, thinning would likely be (indirectly) detectable through the consequent lowered/non-existent growth, leading to lowered/non-existent incremental growth readings from valve movement sensors.

Release of eggs and glochidia

Spawning behaviour often exhibits a pattern on valve movements (Andrade et al., 2016). However, in certain freshwater *Unio* spp., premature release of eggs and immature glochidia larvae (Fig. 1d) can occur

under periods of stress (e.g. reduced oxygen availability, or high temperatures), likely as a mechanism to remove the physiological burden of brooding (which reduces functional gill surface area) (Aldridge & McIvor, 2003; Gascho Landis et al., 2015). This may be a more general stress response, likely susceptible to various environmental pollutants; valve closure (avoidance behaviour) lowers oxygen availability and would likely, depending on the period of closure, elicit gill evacuation to lessen hypoxic stress. The process can also respond directly to certain pollutants. For instance, serotonin and its receptor ligands are important in egg maturation and spawning, and various selective serotonin reuptake inhibitors (used as human antidepressants), e.g. fluoxetine, may be found in sewage effluent and enter surface waters; these can disrupt regulated water uptake and induce spawning (Fong et al., 1996; Fong, 1998; Fong & Molnar, 2008; Cunha & Machado, 2011; Lazzara et al., 2012). Therefore, premature release could inform of pollutant presence, though this metric requires further research to establish expected and abnormal periods of release. Gill evacuation in *Unio* spp. involves a rapid, repeated shunting of the valves (Aldridge & McIvor, 2003) likely discernible through valve movement sensors.

Horizontal movement and burial

Physical activity is another behavioural biomarker which could present an effective tool by which to measure water quality, since it integrates an organism's response to contamination (Kholodkevich et al., 2017). Many bivalves move laterally (Fig. 1e), and vertically (Fig. 1f), both motions typically entailing valve movements. Naturally, species vary; some are completely sessile, whilst others are more mobile, and sensitivity to ambient conditions can affect the necessity of, or inclination towards, movement (e.g. Amyot & Downing, 1997; Peck et al., 2004; Gough et al., 2012; Mitchell et al., 2018). For instance, under drought conditions, species with lower desiccation tolerance track the receding water line more keenly than those less susceptible to desiccation (Gough et al., 2012). Similarly to other behaviours, horizontal movement and burial are likely impacted by chemical stressors, which affect health and impair activity. For instance, cigarette butt leachate, containing a spectrum of toxic compounds, significantly negatively

impacts bivalve burial performance when administered at environmentally-relevant doses (Montalvão et al., 2019). However, activity may not only decrease in response to stressors; prolonged exposure to the antidepressant, fluoxetine, increases lateral and vertical motion (Hazelton et al., 2014). Movement can also provide a means of avoiding stressors, such as desiccation or unfavourable temperatures (Amyot & Downing, 1997; Gough et al., 2012; Mitchell et al., 2018). Therefore, although significantly more research is needed to gauge the utility of this metric, horizontal movement and burial may reflect impacts of perturbations in the environment.

The monitoring of horizontal movement and burial is challenging. This metric is likely most informative when movement is unimpeded. Techniques involving manual measurement of positions (Amyot & Downing, 1997; Mitchell et al., 2018) or manual searching with transmitters and receivers (Gough et al., 2012), would be incompatible with a BEWS. Video or image analysis can provide a tool by which bivalve positions may be tracked (e.g. Zapitis et al., 2021). If automated, perhaps through sophisticated machine learning techniques, this may provide a basis for tracking animal positions in a BEWS. However, the use of such systems would likely be limited to more controlled settings, since the good visibility necessary is unachievable in many field settings.

Alternatively, various biotelemetry solutions, which have been employed with fish, might analogously be employed in bivalves. For instance, acoustic telemetry may be possible; bivalve shells may be equipped with a transmitter (including requisite battery power), that emits low frequency sound signals, and these may be detected by hydrophones. Using multiple hydrophones as receivers, the 3D position may potentially be determined in real-time for equipped bivalves, as for fish (Karp, 2014). Accelerometers can be used alongside such systems to help describe fine-scale movements (e.g. Meese & Lowe, 2020). However, acoustic telemetry applications typically use aquatic life whose movements are of far greater magnitude, and over a far wider geographical range, than those in question (Leclercq et al., 2018; Naughton et al., 2021; Nebiolo & Meyer, 2021). Bivalve motions lie on the scale of < 1 to 10 s of centimetres per day (Amyot & Downing, 1997; Gough et al., 2012; Mitchell et al., 2018), and since positional accuracy of acoustic telemetry typically

falls above this scale (e.g. Meese & Lowe, 2020), and rarely at the sub-metre scale (e.g. Leclercq et al., 2018), it is possible this method may provide positions on too crude a scale to be informative. Furthermore, a further potential challenge is the attenuation of signals that burial would impose in such telemetry set-ups; with a change in medium (sediments to water), reflection of signals at the interface of different media could impair signal strength, as could sediment in itself. There are other potential drawbacks, e.g. the ability of subjects to migrate away from sites of interest, and vulnerability to predation (owing to lack of encapsulation within any housing device). However, assuming these are not such weighty issues, and that tailoring of the approach for the smaller area ranges and positional accuracy requirements of bivalves were possible, such a technique could be compatible with wider BEWS deployment.

Combining measures

Bivalves are sensitive to myriad aquatic pollutants, as may be determined through measurement of the various behavioural metrics identified here. Through combination of different metrics, more nuanced information on bivalve behavioural responses may be gained, potentially allowing ‘fingerprints’ of pollutants to be discerned. For instance, measurement of the valve movement sensitivity to copper revealed the detection limit of the freshwater bivalve, *Dreissena polymorpha*, to be 0.03–0.1 mg/L (Borcherdig, 2006), or possibly as low as 0.01 mg/L (“Detectie limieten - Mosselmonitor”, n.d.). In the marine bivalve, *Mytilus edulis*, this limit may be as low as 0.005 mg/L (“Detectie limieten - Mosselmonitor”, n.d.). The World Health Organisation limit for safe copper levels in drinking water is 2 mg/L (Herschy, 2012), demonstrating the great power bivalves possess as biosensors for water quality. A notable finding from copper exposure experimentation was that the resulting drop in median valve position was a less sensitive indicator than the drop in median heart rate, and the increase in heart rate variability exhibited even greater sensitivity (Curtis et al., 2000). This suggests that finer details in behavioural changes (e.g. heart rate variability) can be particularly useful for detecting pollutants at their lowest concentrations, whilst alterations on an increasingly larger scale (e.g. heart rate or valve position) are more useful in

detecting higher pollutant concentrations. This is consistent with findings comparing measured valve movement attributes; when a stressor was applied to a bivalve (dispersed crude oil to *M. edulis*), mean valve gape was a useful behavioural endpoint at higher pollutant concentrations, whilst distance travelled (a measure of the sum of valve movement in a fixed time interval) proved most sensitive at the lowest concentrations (Redmond et al., 2017). This does not suggest lower value in the less intricate assessments of behaviour; indeed, such differential effects should provide information on the severity (concentration/toxicity) of a pollution episode.

As the examples above highlight, different metrics, and different attributes therein, provide different types of information on bivalve responses to pollutants, each displaying its own sensitivity to different pollutants. Therefore, integration of different metrics/attributes within a BEWS allows enhanced understanding of bivalve responses, increasing the information on pollutants that may be present, and improving system sensitivity. Little has been published on the subject of integrating measures; some work has been undertaken in simultaneous measurement of valve movements and cardiac activity (e.g. Curtis et al., 2000; Andrewartha et al., 2015), and a commercial BEWS, the Biota Guard marine monitoring system, was developed utilising both metrics (Dragsund et al., 2013). Integration with further metrics, e.g. burial or horizontal movement, may reveal further intricacies of behaviour that could improve bivalve-based BEWS practices. Indeed, by combining a range of behavioural measurements, it might be possible to identify toxin-specific ‘fingerprints’, which could prove especially powerful in providing a rapid indication on the nature of a pollution event.

Before integrating technologies, it is important to ensure techniques continue to robustly measure desired metrics, without sacrificing accuracy. For instance, although Hall sensing strategies (to measure valve movements) may potentially measure heart rate simultaneously (at high frequency) (Wilson et al., 2005), and although various cardiac monitoring techniques, e.g. impedance pneumography, can provide indications of valve movements (e.g. Trueman, 1967), unless techniques provide high quality information, simultaneous equipment of bivalves with each separate metric’s technology is best. However, since behaviour can be affected by attachment of devices,

experimentation should ensure that techniques are non-intrusive, i.e. that behaviour is not impacted.

Multi-species monitoring

Commercial BEWSs may not always provide the desired sensitivity. For instance, when freshwater Chinese giant mussels [*Sinanodonta woodiana* (I. Lea, 1834)] were exposed to a variety of chemical pollutants (ranging from chromium to sodium dodecyl sulphate and crude oil) in the Musselmonitor®, very little alarm triggering occurred, even at contamination levels that greatly exceeded those permissible in drinking water (Giari et al., 2017). These tests indicated that, with this species, under these pollutants, the system would have very limited (likely no) useful applications.

Although the unavailing outcome observed in this example may be due to ineffectual setting of alarm thresholds, it is likely that *S. woodiana* lacks the desired sensitivity to the pollutants tested. Sensitivities naturally vary between species; certain pollutants affect different species to different degrees. For instance, although *S. woodiana* caused no alarm triggering with phenol up to 100 mg/L during testing, another freshwater species, *Dreissena polymorpha*, was found (using the Musselmonitor®) to have a detection limit of 14.0 mg/L (“Detectie limieten - Mosselmonitor”, n.d.). Therefore, since high sensitivity to a wide spectrum of pollutants is desirable, it should be beneficial to employ more than one species. Multiple species, deployed simultaneously, may provide better all-round sensing capacity. This should improve the biosensing ability of BEWSs employing bivalves.

Emerging technologies

The most popular techniques for measuring valve movements and cardiac activity are reasonably mature. However, in the constantly evolving world of electronics, on which BEWSs depend, it is easy to envisage many ways that bivalve-based BEWSs could improve in light of such innovation. For instance, smaller and lighter electronic components would lower the likelihood that typical behaviours are encumbered, and render equipment of bivalves for simultaneous measurement of multiple metrics more feasible. The miniaturisation and increased

affordability of components should facilitate equipment of larger samples of bivalves; larger cohorts would improve the quality of information gathered, and render multi-species monitoring more practicable, further enhancing systems. As the quantity of information gathered increases, advances in computational processing and statistical techniques should aid interpretation of data and improve understanding of bivalve responses, improving BEWS sensitivity and informativeness. Furthermore, as batteries become more efficient, remote operation should improve.

One direction that future BEWSs may take is to develop strategies that involve equipping bivalves without the requirement of tethering. Some work has been done creating so-called backpacks for bivalves, containing all the electronic components necessary for measuring behavioural metrics and transmitting collected data, as well as a battery (e.g. Taylor et al., 2013). Untethering allows observation of a more natural mode of life, particularly desirable where mobile species of bivalve are employed. Naturally, equipment weight is significant. Further refinement is necessary, to lower intrusiveness and improve data transmission. However, with electrical innovation, this approach may become far more widespread in future BEWSs. Such a technique would also complement measurement of metrics like horizontal movement and burial.

An alternative approach in future bivalve-based BEWSs is to monitor bivalves without impacting their behaviour or mode of life at all, by removing the necessity for equipping animals. One technology that fulfils this non-intrusive approach is passive acoustics; pioneering work has shown different forms of valve movement produce distinct sounds (Di Iorio et al., 2012; Coquereau et al., 2016), themselves distinct from sounds produced by other benthic organisms, which can be measured by hydrophones over a large area (Di Iorio et al., 2012). The method is applicable to mobile and sessile species, for large cohorts and long-term monitoring. However, the application of this technique in bivalves is little developed; the magnitude of sounds generated is variable, and not all types of movement sounds can always be detected (Di Iorio et al., 2012). Therefore, considerably more research is needed before this technique could be developed for BEWS use.

Accounting for variation at the individual and population level

There are many potential sources of variability in behaviours within bivalves. For instance, cardiac activity can be affected by size and weight of individuals, as well as by age and physiological stresses, like spawning (Xing et al., 2016). Also, whilst behaviours like valve movement can contain various rhythms, which are often highly regulated between different individuals, some individuals can exhibit divergent behaviour (Garcia-March et al., 2016; Comeau et al., 2018). Additionally, responses of bivalves to pollutants depend upon historical exposure; tolerance can develop in a population by selection of certain resistant genotypes, leading to different responses between populations. Furthermore, many bivalves possess inducible “plastic” responses, allowing pollutant tolerance to be modified. For instance, metallothionein, a protein that detoxifies heavy metals, may be upregulated, altering subsequent responses. Such phenotypic alterations have been observed in valve movements and cardiac activity (Bakhmet et al., 2009; Barile et al., 2016; Giari et al., 2017).

There are practical ways to cope with different sources of variation in bivalve behaviours. For instance, desensitisation of cohorts, caused by phenotypic alterations stemming from repeated exposures, renders routine replacement of individuals advisable for maintenance of system sensitivity (Kramer & Foekema, 2001). Consideration of historical exposure is also important when selecting populations to employ, and affords a means of providing high sensitivity (through assembling cohorts from populations with limited pollutant exposure).

The effects of variation between individuals within a bivalve population can be lowered by employing appropriate sample sizes. BEWS cohort size is rarely discussed. However, it is a very significant factor which can severely impact the reliability and sensitivity of systems. Different commercial BEWSs employ different numbers of individuals; the Musselmonitor® employs just eight, whilst the Dreissena-Monitor employs 84, and the Biota Guard marine monitoring system, 32. Differences in choices of sample size are not always apparent. It may be that relatively small samples are appropriate; when an individual’s own behaviour is used as a control, the effect of natural variability lessens (Kramer & Foekema,

2001). Variability effects may be further diminished by highly intensive characterisation of individuals’ behaviours using high frequency data collection, although this necessitates large data sets, and may involve more complex statistical and computational approaches (Jou & Liao, 2006; Schmitt et al., 2011; Sow et al., 2011; Azaïs et al., 2014). However, toxicity testing using pentachlorophenol (a pesticide) revealed detection limits were significantly lower for the Dreissena-Monitor than the Musselmonitor®, despite the considerably simpler (binary) readout of valve gape possible under the former (Borcherding, 1992). The greater sensitivity may be attributed to the markedly larger sample sizes employed by the Dreissena-Monitor, highlighting the importance of ensuring sample sizes are sufficiently large to accommodate the innate variability of individuals. This recommends thorough testing of innate specimen variability, to ensure that the number of specimens used allows reliable results to be generated, which are independent of the number of individuals employed. For instance, prior testing with the Dreissena-Monitor indicated a cohort of at least 24 individuals was necessary (Borcherding, 1992). A potential means of establishing appropriate population sizes for a species (i.e. number of replicates) is to determine the number of specimens required to give a coefficient of variation (ratio of standard error to arithmetic mean) of 0.2 at the most, which is considered a reasonable error in most benthic samples (Elliott, 1971; Aldridge et al., 2007).

Other than simply controlling certain parameters, e.g. size/age/sex of individuals, another means of reducing variation between individuals used in BEWSs is to employ specimens from a known clonal lineage. This technique is employed in biomonitoring strategies involving the water flea, *Daphnia magna* Straus, 1820 (Shaw et al., 2008), and is a viable option for bivalves; for instance, the Asian clam, *Corbicula fluminea*, possesses one genotype across Europe, making it possible to control for genotype. However, it is important to remember that this is an invasive species, and use of such species should be discouraged, owing to the threat invasives pose to native flora and fauna. Various BEWSs do use invasive species, such as the Dreissena-Monitor (*Dreissena polymorpha* is invasive in Europe), but such species should only be used when there is no risk of escape (both directly and through reproduction) or

Table 1 Summary of the valve movement and cardiac responses observed in bivalve molluscs when exposed to toxicants, along with key advantages and disadvantages of using these metrics as biosensors of water quality

Established metrics			
Metric	Response	Advantages	Disadvantages
Valve movements	Closing of the valves for a fixed period of time (avoidance behaviour) Flapping, i.e. rapid cycles of abduction (valve-opening) and adduction (valve-closing) Opening valves progressively narrower (indicating lowering or cessation of the pumping)	Extensively studied in laboratory and field, and under various physical and chemical conditions Allows sub-lethal detection of water pollutants (e.g. Borcherd-ing, 2006) Dose- (e.g. Liu et al., 2016) and time-dependent (e.g. Tran et al., 2003) responses can be characterised Certain pollutants can induce specific valve movement patterns (Andrade et al., 2016)	High variability within and between individuals and species Rhythms present in many species (e.g. Garcia-March et al., 2016) Sensitive to various ambient factors, making measurement of local physical conditions sensible Closures can be lengthy, limiting interim utility (Kramer & Foekema, 2001)
Cardiac activity	Heart rate alteration Inter-pulse duration change Heartbeat can adopt different patterns, such as “burst activity”, or entirely irregular patterns Heartbeats can cease (temporary cardiac arrest) Heart amplitude (strength of contraction) alteration	Extensively studied in laboratory and field, and under various physical and chemical conditions Allows sub-lethal detection of water pollutants (e.g. Dragsund et al., 2013) Dose- and time-dependent responses can be characterised (e.g. Curtis et al., 2000) Rapidly impacted by toxic substances (Nicholson, 1999a)	High variability within and between individuals and species Rhythms present in many species (e.g. Bakhmet & Zdorovenov, 2010) Sensitive to various ambient factors, making measurement of local physical conditions sensible Traces can be difficult to interpret (e.g. Seo et al., 2016) Temporary arrest can occur unpredictably (e.g. Bakhmet & Khalaman, 2006) Heart amplitude measurement often unreliable

when the species is already established. Furthermore, there is emerging evidence that translocation of native bivalves risks introduction of harmful pathogens into the recipient ecosystem (Brian & Aldridge, 2019; Brian et al., 2021). The species deployed in BEWSs should ideally always be local and native (Kramer & Foekema, 2001).

Conclusions

As predominantly sessile, ubiquitous, and easy-to-handle organisms, sensitive to a broad spectrum of water pollutants, bivalves offer an effective tool as biosensors of water quality. Extensive research has proven their value in bioindication and bio-monitoring strategies, promoting interest in their

employment as BEWS sensing organisms. Different behaviours may be used as metrics in such BEWSs; focus has been on valve movements and cardiac activity, which have their advantages and disadvantages (as summarised in Table 1). A wide variety of technologies have been developed to monitor these metrics, including some commercial BEWSs. However, this narrow focus has ignored other aspects of bivalve behaviour, e.g. burial and egg/glochidia release, which research suggests may exhibit sensitivity to perturbations, like pollutants. These alternative metrics may, through advancements, offer novel glimpses into bivalve ecology and provide other bases for BEWS development, potentially using alternative technologies (Table 2).

The various metrics may independently provide extensive information, necessary for sensitive

Table 2 Summary of the ways that alternative metrics of bivalve behaviour may respond to toxicants and how they may be measured in the field

Metric	Response	Potential technique for measurement in the field	Explanation
Bivalve growth	Shell growth rate can increase or decrease depending on how favourable ambient conditions are Dissolution, from prolonged valve closure under unfavourable local conditions, can decrease shell thickness	The same techniques developed to monitor valve movements may be employed to measure bivalve growth, as well as potentially dissolution events and release of eggs/glochidia	As growth, originating in the mantle cavity, leads to incremental increases in the maximum/minimum opening of valves, a readout of growth can be gained (Wilson et al., 2005; Schwartzmann et al., 2011) Dissolution decreases shell thickness (e.g. Nagarajan et al., 2006), and may thereby bring valves closer together, causing a decrease in maximum/minimum opening values; alternatively, a check in growth (read by constant valve distance) may occur
Release of eggs and glochidia	Eggs/glochidia release can take place prematurely under stressful conditions to relieve gills of the physiological burden of brooding The direct action of certain pollutants can stimulate premature egg/glochidia release		Gill evacuation is marked by repeated shunting of the valves (Aldridge & McIvor, 2003), detectable through valve movement sensors
Horizontal movement and burial	Lateral and vertical motion can be employed as a means to escape, or limit exposure to, unfavourable ambient conditions Efficiency of lateral and vertical motion reflects the health of individuals, which reflects ambient conditions Certain pollutants may also stimulate physical activity	Biotelemetry techniques, such as acoustic telemetry, involving deployment of transmitters (emitting low frequency sound signals) on animals, and hydrophones in the waterways (to detect these signals), may be a means to observe these motions, and how they alter, through time	The tendency of individuals to move horizontally or vertically, and the efficiency with which this is achieved, requires remote detection of animal positions Such biotelemetry techniques (e.g. Leclercq et al., 2018; Meese & Lowe, 2020) may, with tailoring of systems, allow tracking of individual bivalve 3D locations through time, informing of these movements

BEWSs. However, combination of these metrics, and different attributes within them, has the potential to deliver greater understanding of responses, providing more intricate details and likely improving system sensitivity. In addition, simultaneous deployment of multiple bivalve species, each with different sensitivities, should help provide better all-round biosensing abilities in bivalve-based BEWSs. The continuing evolution of the world of electronics, generating smaller and cheaper components, increases the feasibility of multiple metric measurement and larger, multi-species cohorts in BEWSs. As the quantity of information generated increases, advancements in computing and statistical approaches should aid data processing and more sophisticated alarm triggering. Future directions may entail use of untethered, or entirely unequipped, animals. Provided that sources of variation, both at the individual and population level are considered and controlled for as far as practical, BEWSs employing bivalves have great power. The scope for new developments in the area of bivalve-based BEWSs is significant, and this field has the potential to provide important social, economic and environmental benefits, from improving future management of water quality to improved aquaculture practices and conservation of aquatic ecosystems.

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Data availability The search criteria used to collate data for the systematic reviews (presented in Figs. 2 and 3) are provided within their respective captions. The data thereby extracted are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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