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USE OF HIGH-FREQUENCY NONINVASIVE ELECTROMAGNETIC BIOSENSORS TO DETECT OCEAN ACIDIFICATION EFFECTS ON SHELLFISH BEHAVIOR

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ABSTRACT Although ocean acidification studies related to marine animal behavior have increased in recent years, the behavioral effects of ocean acidification on shellfish are relatively understudied, even though marine shellfish exhibit a wealth of behaviors that can modify organismal interactions and biological community functioning. Furthermore, detecting acute behavioral changes may provide a biological indicator of ecosystem stress and/or an early warning system for aquaculture operations. This article highlights a new and emerging technology—high-frequency noninvasive (HFNI) electromagnetic biosensors—that can be used to document acute and long-term effects of elevated CO₂ on the valve-gaping behavior of marine bivalves. An overview of the technology is presented, and the current and potential uses of these biosensors in ocean acidification research are highlighted, along with current limitations and next steps. Although a handful of studies have used these biosensors to test for effects of acidification on bivalve valve-gaping behavior, their potential for testing critical and novel hypotheses regarding ocean acidification effects in a broader range of shellfish taxa is currently underused. Ultimately, this article provides a basis for expanding ocean acidification research on shellfish behavior through the use of HFNI electromagnetic biosensors.

KEY WORDS: animal behavior, environmental stress, global change, pH, shellfish health, valvometry

INTRODUCTION

Shellfish are thought to be particularly vulnerable to ocean acidification because of their reliance on calcium carbonate skeletons, which, for many (but not all) species, may dissolve and/or be more difficult to make in a more acidified ocean (Orr et al. 2005, Gazeau et al. 2007, Hoegh-Guldberg et al. 2007, Ries et al. 2009, Hoffmann et al. 2010). As such, numerous studies have documented the effects of ocean acidification on shellfish calcification and physiology (Gazeau et al. 2013, Kroeker et al. 2013). Relatively fewer studies, however, have focused on the effects of ocean acidification on shellfish behavior (Clements & Hunt 2015).

The initial phenotypic response of many marine organisms to environmental stressors, including ocean acidification, is to alter their behaviour to avoid lethal effects, or to avoid the associated metabolic costs of maintaining physiological balance, under a given stressor (Schreck et al. 1997). For example, juveniles of the cod *Gadus morhua* actively avoid high-CO₂ seawater presumably to avoid potential lethal effects (Jutfelt & Hedgärde 2013). In shellfish, the intertidal snail *Littorina littorea* is reported to alter its behavior to avoid the lethal effects of predation when inducible shell thickening is disrupted by ocean acidification (Bibby et al. 2007). Infaunal clams tend to reject burrowing into acidified sediments to avoid “death by dissolution” (see Clements & Hunt 2017 for review). When unable to avoid more acidified conditions, exposure to ocean acidification can affect various other behaviors in shellfish as well. For example, general activity levels are reported to be affected (Dissanayake & Ishimatsu 2011, Alenius & Munguia 2012, Spady et al. 2014, Queirós et al. 2015). Responses of shellfish prey to predators can also be affected (Manríquez et al. 2013, Appelhans et al. 2014,

Manríquez et al. 2014, Watson et al. 2014; also see Clements & Comeau 2019 in this issue), along with shellfish predator responses to prey (Appelhans et al. 2012, Landes & Zimmer 2012, Manríquez et al. 2014, Quierós et al. 2015) and grazer responses to food (Burnell et al. 2013, Barry et al. 2014).

Behavioral effects of ocean acidification, as described earlier, have the capacity to influence ecosystem structure and function through altered biological interactions (Nagelkerken & Munday 2016); thus, understanding the effects of ocean acidification on shellfish behavior is imperative for understanding the ecological effects of this global change stressor. Behavioral alterations under stressful conditions can also result in biological trade-offs for a given individual, as time and energy spent engaging in altered behaviors often come at the cost of other key biological functions, such as feeding, reproduction, metabolic activity, and immune defense (Loose & Dawidowicz 1994, Rawlings 1994, Werner & Hall 1988, Rigby & Jokela 2000). Furthermore, the detection of acute behavioral changes in temporary periods of seawater acidification may serve as an early warning biological indicator of ecosystem stress, as behavioral alterations such as increased general activity or alterations in bivalve gaping behaviors can be indicative of environmental stress (Akberali & Trueman 1985, Andrade et al. 2016). Thus, real-time behavioral observation could potentially provide a bio-monitoring tool for coastal shellfisheries in the wake of ocean acidification and other marine global change stressors (Andrade et al. 2016).

Given the importance of understanding the impacts of coastal and open ocean acidification on shellfish behavior from both economic and ecological perspectives, this article highlights a new and emerging technology—high-frequency noninvasive (HFNI; after Andrade et al. 2016) electromagnetic biosensors—for detecting the behavioral effects on shellfish. A general overview of the technology and how it is currently used (i.e., in studies measuring bivalve gaping behavior) is provided, and the handful of ocean acidification

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studies to date that have used this technology for such purposes are reviewed. A number of potential uses of this technology for testing novel and important hypotheses regarding ocean acidification effects on shellfish behavior are provided along with key limitations and next steps.

CONTEMPORARY USE OF HFNI ELECTROMAGNETIC BIOSENSORS

From a shellfish behavior perspective, HFNI electromagnetic biosensors have been strictly used in “valvometry” studies with bivalves. Valvometry can be defined as the measurement of gaping behavior (closing and opening of the valves) of bivalve molluscs. The origin of valvometry can be traced back a century ago when Nelson (1921) reported that “an apparatus was designed to register the shell movements of the oyster.” Up to the 1970s, this mechanical approach generated several thousand records of shell movements (Galtsoff 1964). This mechanistic approach to valvometry was gradually abandoned with the advent of electronics. This transition began with Higgins (1980) cementing a nylon line onto the upper valve of an oyster and relaying that line to an electronic strain gauge recorder. The appearance of HFNI electromagnetic biosensors subsequently attracted new interest in valvometry, prompting an exponential growth in the number of valvometry articles (Fig. 1).

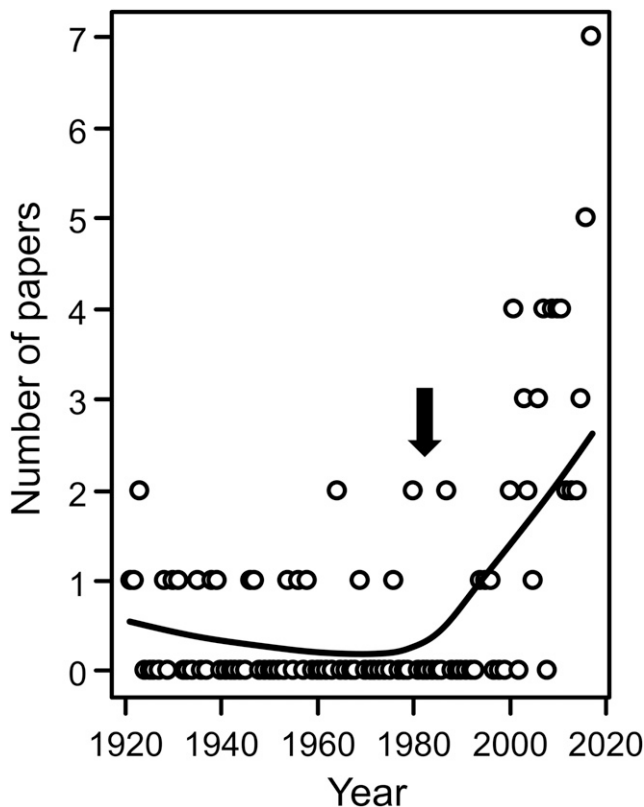


Figure 1. Distribution frequency ($n = 78$) of articles integrating valvometry over the past century. Arrow indicates the approximate time of appearance for HFNI electromagnetic biosensors. The best fit line is a locally weighted scatterplot smoothed curve.

There are two major kinds of electromagnetic principle-based sensors that measure valve gaping, which are reviewed by Jou et al. (2013). Both kinds require gluing or cementing small devices at the margin of each valve. A Hall effect-based device will measure the magnetic field (flux density) between a Hall effect sensor cemented on one valve and a magnet cemented on the other valve (Fig. 2A), which changes in relation to the distance between the magnet and sensor and, thus, the two valves (Fig. 2B) (Nagai et al. 2006). This magnetic flux density can be converted into voltage values using a dynamic-strain recording device; voltage values can then be translated into a millimeter distance between the two valves, and further translated into a size-standardized gape measure (gape angle in $^{\circ}$) (Wilson et al. 2005, Comeau 2014). From this, a number of biologically relevant measures of activity can be derived (e.g., total activity, time spent open versus closed, and mean distance of opening). Another approach is based on electromagnetic induction between two electric coils (Tran et al. 2003). More recent engineering initiatives are transforming such technology into fully automated and field-deployable monitoring devices. Some of these new sensors integrate real-time monitoring capabilities in conformity with the concept of biological early warning systems (Kramer & Foekema 2001, Borcharding 2006). The MolluSCAN eye, for instance, is a highly specialized valvometry system that automatically transfers real-time data on valve movements of sentinel bivalves through a mobile network (Andrade et al. 2016), allowing land-based servers to scan for abnormal behavior.

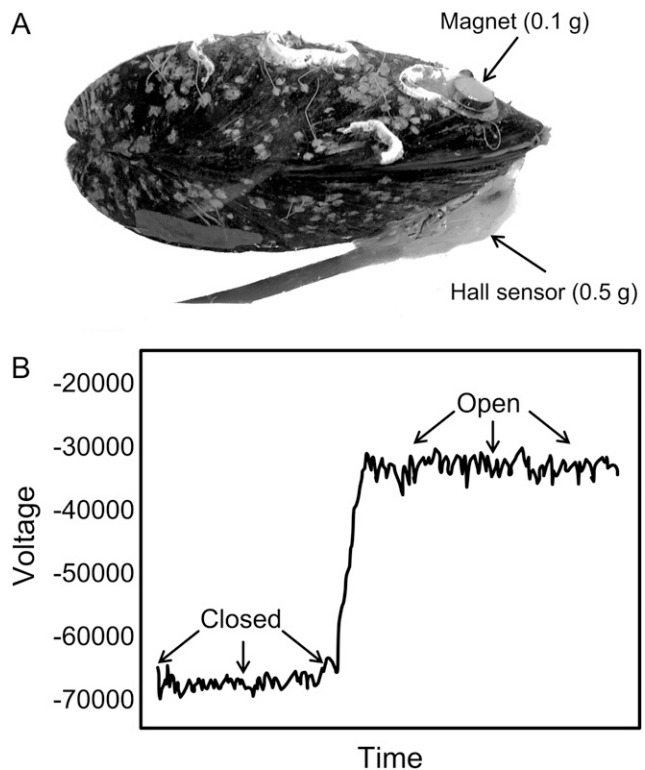


Figure 2. (A) Depiction of the Hall effect sensor and magnet setup on a blue mussel (*Mytilus edulis*). (B) Hypothetical example of voltage data (in μV) provided by Hall effect-type biosensors depicting μV values when a bivalve is open (i.e., larger distance between the sensor and magnet) and closed (i.e., smaller distance between the sensor and magnet).

A REVIEW OF HFNI ELECTROMAGNETIC BIOSENSORS IN THE CONTEXT OF OCEAN ACIDIFICATION STUDIES ON SHELLFISH BEHAVIOR

To date, only a handful of studies have used HFNI electromagnetic biosensors to test for the effects of elevated CO₂ on bivalve valve-gaping behavior (Table 1). In a laboratory experiment simulating sub-seabed CO₂ leakage, Bamber and Westerlund (2016) reported no effects of elevated CO₂ on valve-gaping behavior in *Arctica islandica* until seawater pH reached ~6.20, at which gaping activity (i.e., consecutive opening and closing of the valves) markedly increased; however, the gaping behavior returned to normal levels (i.e., levels comparable to control animals) after 5 days of exposure to pH 6.20. In a more recent laboratory experiment using Hall effect biosensors, Clements et al. (2018) found that a 9-day exposure to elevated CO₂ up to 8,000 µatm did not impact the valve-gaping response of *Crassostrea virginica* to acute, short-term temperature increases (Δ ~20°C; 10°C → 30°C). Furthermore, although electromagnetic sensors were not used to measure valve-gaping behavior, Jakubowska and Normant (2015) documented no effect of elevated CO₂-induced acidification (pH

7.5 and 7.0 compared with a control of pH 8.1) on the valve-gaping behavior of mussels (*Mytilus edulis*).

Although not directly comparable to CO₂-induced ocean acidification studies, other studies have documented effects of seawater pH and freshwater pCO₂ on bivalve gaping behavior, which provide relevant information on pH and CO₂ responses. Comeau et al. (2017) reported that valve opening in mussels (*Mytilus edulis*) decreased as seawater pH increased from 8.0 to 9.0 (using calcium hydroxide to buffer seawater pH). Hasler et al. (2017) also documented the effects of elevated pCO₂ on the valve-gaping behavior of three freshwater mussel species from North America, reporting species-specific responses. In an experiment acutely exposing two freshwater bivalves to elevated CO₂ (incremental increases of ~14,000 µatm every 5 min), Hasler et al. (2017) reported acute valve closures in *Pyganodon grandis* and *Lampsilis siliquoidea* at ~200,000 and ~80,000 µatm, respectively. In a separate experiment exposing three species of mussels to elevated CO₂ over 2 days, Hasler et al. (2017) found that *Lampsilis cardium* remained open for nearly the entire duration of the CO₂ exposure, whereas *P. grandis* exhibited no response during the exposure period, but

TABLE 1.

Summary of studies using electromagnetic biosensors to test for effects of pH/CO₂ on valve-gaping behavior in marine and freshwater bivalves. pCO₂ is reported in µatm.

Reference	Species	Exposure time	High pCO ₂	Δ pCO ₂	Low pH	Δ pH	Response
Marine							
Bamber and Westerlund (2016)	<i>Arctica islandica</i>	Gradual pH decrease over 11 days	–	–	7.03	1.05	No effect
			–	–	6.81	1.27	No effect
			–	–	6.62	1.46	No effect
			–	–	6.42	1.66	No effect
			–	–	6.24	1.84	Increased valve movements at lower pH
–	–	6.06	2.02	Increased valve movements at lower pH			
Comeau et al. (2017)*	<i>Mytilus edulis</i>	Gradual pH increase (from 8.0 to 9.0) over 3 h	–	–	8.00	1.00	Increased valve opening at lower pH
Clements et al. (2018)	<i>Crassostrea virginica</i>	9 days	7,423–8,367	6,355–7,299	6.79–6.90	0.78–0.89	No effect on response to acute temperature ↑
			4,570–6,508	3,502–5,461	6.91–7.08	0.60–0.77	No effect on response to acute temperature ↑
			2,331–2,431	1,263–1,362	7.30–7.34	0.34–0.38	No effect on response to acute temperature ↑
			1,670–1,721	602–652	7.44–7.45	0.23–0.24	No effect on response to acute temperature ↑
Freshwater							
Hasler et al. (2017)	<i>Lampsilis cardium</i>	2 days	69,430	69,330	6.60	1.90	Remained gaping during the CO ₂ exposure period
	<i>Lampsilis siliquoidea</i>	2 days	72,551	72,451	6.60	2.10	Time spent gaping unaffected; number of valve movements decreased 40-fold
	<i>Pyganodon grandis</i>	2 days	69,430	69,330	6.60	1.90	Acute valve closure at ~80,000 µatm
							Time spent gaping increased after the CO ₂ exposure period
							Acute valve closure at ~200,000 µatm

* Used calcium hydroxide to increase seawater pH rather than CO₂ to decrease pH.

increased gaping (i.e., decreased time closed) after the exposure. In addition, the time spent gaping in *L. siliquioidea* was unaffected by elevated CO₂, but the number of valve movements decreased 40-fold during CO₂ exposure (Hasler et al. 2017).

Ultimately, studies to date suggest that bivalve valve-gaping behavior is insensitive to elevated CO₂ in the context of ocean acidification. The number of such studies is grossly limited, however, and current studies have used species known to be tolerant to a wide range of environmental conditions, including CO₂. Thus, studies testing similar hypotheses in other bivalve species are warranted.

EXPANDING THE UTILITY OF HFNI ELECTROMAGNETIC BIOSENSORS IN OCEAN ACIDIFICATION–SHELLFISH BEHAVIOR RESEARCH

The Functional Role of Valve Gaping under Ocean Acidification

Although studies assessing valve-gaping behavior under ocean acidification suggest that this is an insensitive behavioral trait, these studies simply measure basal valve-gaping activity and fail to place this behavior in a functional context. Valve-gaping behavior has been linked to a number of functional processes that are known to be impacted by ocean acidification. For example, gaping behavior has been linked to physiological rates in marine bivalves (e.g., Markich et al. 2000, Riisgård 2001, Frank et al. 2007), although such relationships are highly inconsistent in the literature and a lack of relationship between valve gaping and physiological rates is often reported (Newell et al. 2001, Maire et al. 2007). Thus, coupling HFNI biosensor valvometry with other biosensors designed to measure physiological rates (e.g., heart rate [Fedotov et al. 2000] and glucose [Kim et al. 2015] sensors) could provide information on the physiological role (or lack thereof) of valve gaping under ocean acidification conditions. Valve gaping is also thought to play a role in predator avoidance (Robson et al. 2007, 2010, Carroll & Clements 2019), and a number of studies have reported that shellfish responses to predators can be affected by exposure to ocean acidification conditions (Manriquez et al. 2013, Appelhans et al. 2014, Manriquez et al. 2014, Watson et al. 2014; also see Clements & Comeau 2019 in this issue). Similarly, valve “microclosures” are thought to play an important role in mitigating the effects of toxic algae on bivalves (Basti et al. 2009, Tran et al. 2010, 2015), and ocean acidification could impact bivalves’ ability to use microclosures in response to toxic algae, just as it could affect predator avoidance valve closures. Thus, although ocean acidification may not affect basal valve-gaping activity, it has the potential to impact the functional roles of valve gaping. The potential effects of ocean acidification on valve gaping in a functional context remain undocumented, however, and warrant future research.

Behavioral Effects of Ocean Acidification in the Context of Additional Stressors

It is well known that the effects of ocean acidification on marine biota are unlikely to occur in isolation but will occur alongside other environmental changes, such as ocean warming, deoxygenation, eutrophication, and salinity changes (Harvey et al. 2013, Breitburg et al. 2015, Baumann & Gobler 2016). Likewise, direct activity of humans on the water can exert additional stressors never before experienced by marine organisms

(e.g., contaminants and noise pollution; Pipe & Coles 1995, Guéguen et al. 2011, Peng et al. 2015, de Soto 2016). Consequently, multiple stressor studies using long-term exposures to ocean acidification under varying levels of additional stressors are warranted to better understand how ocean acidification will impact marine fauna in the future. Indeed, studies have tested for the effects of other environmental stressors on valve-gaping activity in bivalves. For example, using electromagnetic biosensors, Porter and Breitburg (2016) documented the effects of diel-cycling hypoxia (reduced oxygen) on *Crassostrea virginica*, reporting that oysters tended to close under severe exposure to hypoxia. In addition, Ballesta-Artero et al. (2017) suggested that valve gaping in *Arctica islandica* appears to be influenced by food availability (a key variable of concern in ocean acidification studies; e.g., Brown et al. 2018), temperature, and photoperiod. Studies have also documented the effects of contaminants (Tran et al. 2003, 2004, Liao et al. 2009, Comeau et al. 2017) and noise pollution (Charifi et al. 2017) on the behavior of marine bivalves using electromagnetic biosensors. Such studies, however, have not tested for such effects in the context of ocean acidification. Given the importance of understanding ocean acidification effects in the context of other stressors, studies assessing the cumulative impacts of ocean acidification and other stressors as mentioned earlier are warranted.

In situ Field Measurements

A critical limitation of ocean acidification research until now has been the heavy emphasis on laboratory experiments and a lack of field studies. With the exception of recent studies at CO₂ vents, few ocean acidification studies use field experiments to study effects on animal behavior (Clements & Hunt 2015). Furthermore, direct correlations between animal behavior and carbonate system variability in the wild are currently undocumented. One highly beneficial aspect of HFNI electromagnetic biosensors is that they can be deployed in the field for relatively long periods of times (Lorenz et al. 2012, Ballesta-Artero et al. 2017, Comeau et al. unpublished data). Thus, these sensors have the capability to drastically expand ocean acidification–shellfish behavior research from the laboratory into the field. Such an approach provides a basis for studies exposing animals to elevated CO₂ in the laboratory and subsequently measuring behavior directly in the field. Furthermore, these biosensors open the possibility to directly correlate shellfish behavior with variable conditions in coastal systems by coupling abiotic monitoring with behavioral measurements using these sensors. Such studies would not only be restricted to correlations with carbonate system parameters but can also incorporate a wealth of abiotic and biotic variables. This approach can greatly increase the ability to understand how ocean acidification and global change stressors impact shellfish behavior and the associated ecological ramifications of such effects.

Other Behaviors and Taxa

As previously mentioned, the use of HFNI electromagnetic biosensors to date has been focused on measuring valve-gaping behavior in bivalves. At their core, however, the data generated by these biosensors simply provide a proxy for the distance between two points and allow for the determination of various associated behavioral metrics in a wide range of shellfish taxa

that can be impacted by ocean acidification (see Fig. 3 for examples). For example, one could theoretically measure burrowing behavior in marine bivalves (which is known to be affected by elevated CO₂; Green et al. 2013, Clements & Hunt 2014, Rodríguez-Romero et al. 2014, Clements et al. 2016, 2017, Clements & Hunt 2017) using these biosensors by fixing the sensor on a stable structure just above the sediment surface and attaching the magnet to the edge of the bivalve shell near the siphon(s). Such an approach would not only indicate the time needed to burrow and the burrowing depth, but could also shed light on the movements of bivalves within their burrows on short time scales. In gastropods, electromagnetic biosensors could also be used to measure boldness and/or stress recovery by placing a magnet on the snail's operculum and a sensor on the shell; the signal intensity could then be used to measure the time it takes for snails to emerge after a stressful event (e.g., attempted predator attack). In crustaceans, claw activity could be measured using these sensors, and measures of claw movements can be generated using similar metrics to bivalve valve gaping (e.g., total activity, time with claw open, and average claw movement). Nearest-neighbor distances could also be measured by placing a sensor on one individual and a magnet on the other. Ultimately, HFNI electromagnetic biosensors

have the capacity to measure various behaviors in a wide range of shellfish taxa under ocean acidification. Thus far, however, these sensors have been underused to this regard, and future research is warranted.

THE ROLE OF HFNI ELECTROMAGNETIC BIOSENSORS FOR MITIGATION OF OCEAN ACIDIFICATION IMPACTS ON SHELLFISH AQUACULTURE

A key component of ocean acidification mitigation in aquaculture is the early detection of corrosive and detrimental conditions; once detected, such conditions can be combated in various ways (Clements & Chopin 2016). It has been recently proposed that HFNI biosensors measuring valve gaping may be useful as biomonitoring tools in marine systems and for areas of marine development (e.g., oil rigs and aquaculture sites) (Andrade et al. 2016). With respect to using HFNI sensors to monitor for ocean acidification effects at aquaculture sites, studies to date suggest that this may not be a viable option, as valve-gaping responses of bivalves appear insensitive to elevated seawater CO₂, with the exception of extreme conditions (pH <6.5). The number of such studies is limited, however, and more research on other bivalve species is warranted. Furthermore, expanding this technology to different behaviors in shellfish taxa may render this technology useful in aquaculture monitoring for ocean acidification effects.

TECHNOLOGICAL LIMITATIONS

Although HFNI electromagnetic biosensors have the potential to greatly advance ocean acidification research on shellfish behavior, this technology, as with any technology, comes with limitations. One major limitation is the size of the sensors. Given their size (Fig. 2A), the use of these sensors is restricted to adult or large juvenile individuals. Given that larval individuals are considered to be most susceptible to the effects of ocean acidification (e.g., Kroeker et al. 2010, 2013), particularly for functions sometimes related to valve gaping (e.g., feeding rates; Clements & Darrow 2018), these biosensors are currently unable to detect ocean acidification effects on the most sensitive life stages of shellfish. Efforts to reduce the physical size of these biosensors can help in their application to smaller sized individuals and ultimately enhance their utility in ocean acidification studies and beyond.

As previously mentioned, one major benefit of HFNI electromagnetic biosensors is the ability to deploy them in the field. Although this holds true for the technology in its current state, some limitations apply here. For example, submersing this technology underwater is an issue, particularly from a cost-benefit perspective when one considers the financial cost (upward of \$10,000 for the most expensive systems, although custom systems can be created using open-source platforms such as Arduino for far cheaper prices, i.e., hundreds of dollars) of the equipment and the high risk of damaging it by submersing it in water. Furthermore, the distance from shore at which these sensors can be deployed is also limited because data must be retrieved fairly regularly. As such, the use of these biosensors in the field is limited to relatively shallow depths in nearshore environments. Thus, technological advances (e.g., submersible wireless data collection and satellite data relaying) could increase the scope of utility for these biosensors in the field.

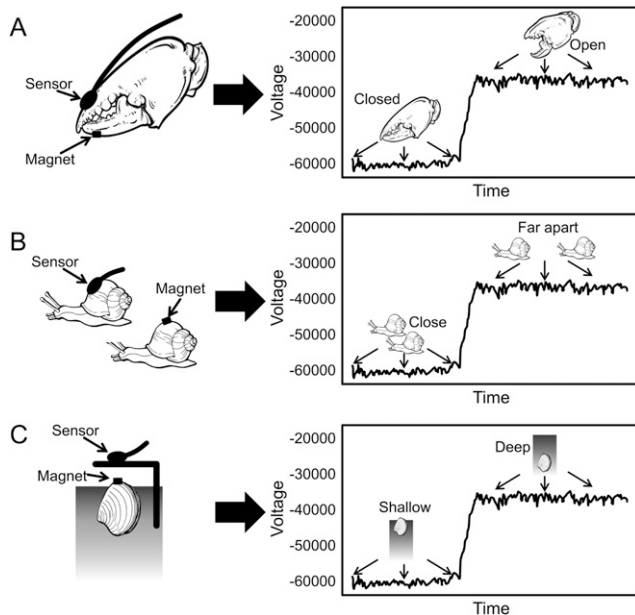


Figure 3. Hypothetical examples of additional behaviors (aside from bivalve valve gaping) in various taxa that can potentially be assessed using HFNI electromagnetic biosensors (Hall effect-type biosensors are depicted) including crustacean claw movements (A), nearest-neighbor distances (B), and bivalve burrowing behavior (C). Graphs depict hypothetical voltage data (in μV) provided by Hall effect-type biosensors depicting μV values when the sensor and magnet are close (lower μV) and farther apart (higher μV). Studies have yet to use these sensors for such behaviors, and future research focused on developing biosensors for behaviors aside from bivalve valve gaping is warranted. Crab claw images reprinted with permission from Pinterest user “BlackAkaliko.” Snail image was obtained online from “Get Drawings” and is used under a Creative Commons CC-BY 4.0 license (<http://getdrawings.com/snail-drawing#snail-drawing-9.jpg>). Clam image was obtained online from the free clipart site “Pngfly” at <https://www.pngfly.com/png-35qff/> and was created by user “Erlineka20.”

Finally, data handling and processing is also a potential issue with using these sensors. The sheer volume of data can be overwhelming, given that the measurement frequency for a single sensor can be less than 1 sec. Furthermore, data handling techniques are predominantly manual at present. Thus, handling the data and deriving meaningful measurements of behavior can be difficult. As such, efforts to optimize and automate data handling and the derivation of meaningful behaviors are warranted.

CONCLUSIONS

High-frequency noninvasive electromagnetic biosensors have greatly aided in studies of bivalve valve-gaping behavior and the drivers that can influence it. With respect to elevated CO₂, a limited number of studies using these biosensors have reported that valve gaping is insensitive to drastic increases in seawater CO₂. These studies, however, used highly tolerant species, and additional studies with other species are warranted. In addition, although research has assessed both elevated CO₂ and other stressors in isolation, the effects of ocean acidification on valve gaping in the context of multiple stressors have yet to be studied. Although these biosensors have proven useful in

measuring bivalve gaping, they have the capacity to measure numerous other behaviors in various shellfish taxa, in both the laboratory and field, and the development of such techniques should, thus, be a research priority. Finally, although HFNI electromagnetic biosensors come with limitations, technological innovations to reduce cost and sensor/magnet size and increase field capabilities and data handling efficiency will enhance the utility of these biosensors. Ultimately, much more research into the effects of ocean acidification on shellfish behavior is warranted, and HFNI electromagnetic biosensors are a promising tool for such investigations.

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