A simplified biomimetic temperature logger for recording intertidal barnacle body temperatures

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Abstract

Monitoring the thermal environment and responses of indicator species is fundamental for understanding and predicting ecological consequences of ongoing and future environmental changes. With the recent development of miniaturized temperature sensors (e.g., iButtons), which can be incorporated into biomimetic loggers (e.g., robolimpets), it is possible to routinely obtain long-term estimates of body temperatures of intertidal organisms. A crucial step in the assembly of these biomimetic devices involves removing the circuit board from an iButton, a process which is not simple and often results in damage to the electronics or loss of calibration. In this study, we describe a simplified process to assemble biomimetic loggers, and use it to build robobarnacles (mimicking tropical and subtropical barnacles of the genus *Tetraclita*). The process involves copper-coating the stainless steel case of iButtons through an electroplating process, allowing solder joints to be made to the surface casing, thus avoiding opening the iButton to make a connection with its circuit board. This approach makes the manufacturing process simpler, faster, and prevents calibration loss, but is only suitable for species large enough to accommodate a complete iButton.

Introduction

Global climate change has been increasingly associated with important alterations in marine ecosystems including increases in species' mortality due to heat stress (Jones et al. 2009), range shifts, local extinctions and invasions (Herbert et al., 2003; Mieszkowska et al. 2006, 2014; Sousa et al. 2012), and consequent changes in ecosystem function (Thompson et al. 2002; Hawkins et al. 2008). In this context, monitoring the thermal environment and the thermal responses of indicator species is essential to understand and predict the ecological consequences of these changes (Helmuth et al. 2006; Mieszkowska et al. 2006). The intertidal zone is an excellent model system to study the effects of global warming because intertidal species have to cope with dynamic variation in environment between terrestrial and marine conditions every day, making them particularly sensitive to thermal stress (Helmuth et al. 2006; Hawkins et al. 2008, 2009). As most intertidal species are ectothermic, large variations in temperature have a great influence on their physiology, growth rates, reproductive output, and survival (Southward 1958; Hines 1978; Zwaan and Mathieu 1992; Roberts et al. 1997).

Although the body temperatures of ectotherms are strongly affected by environmental temperatures, they are also influenced by individual morphology, size, color, behaviour and thermal capacity of body fluids (Porter and Gates, 1969; Porter et al., 1973; Hertz et al. 1993; Helmuth, 1998). The body temperatures of ectotherms are, therefore, often different from the ambient air or substratum surface temperatures. In the intertidal zone, many organisms display body temperatures that are different from those measured in air or from adjacent rock surfaces during low tides (Williams and Morritt 1995; Chan et al. 2006). As result, air and rock temperatures are often poor predictors of body temperatures of intertidal species (Helmuth and Hofmann 2001) and may not reflect the actual physiological conditions of the organism.

In order to determine thermal stress levels, researchers have started adopting in situ measurements of intertidal animal temperatures (e.g., barnacles and limpets, Lewis 1963; Wolcott 1973; Williams and Morritt 1995; Fitzhenry et al. 2004; Chan et al. 2006). With the development of

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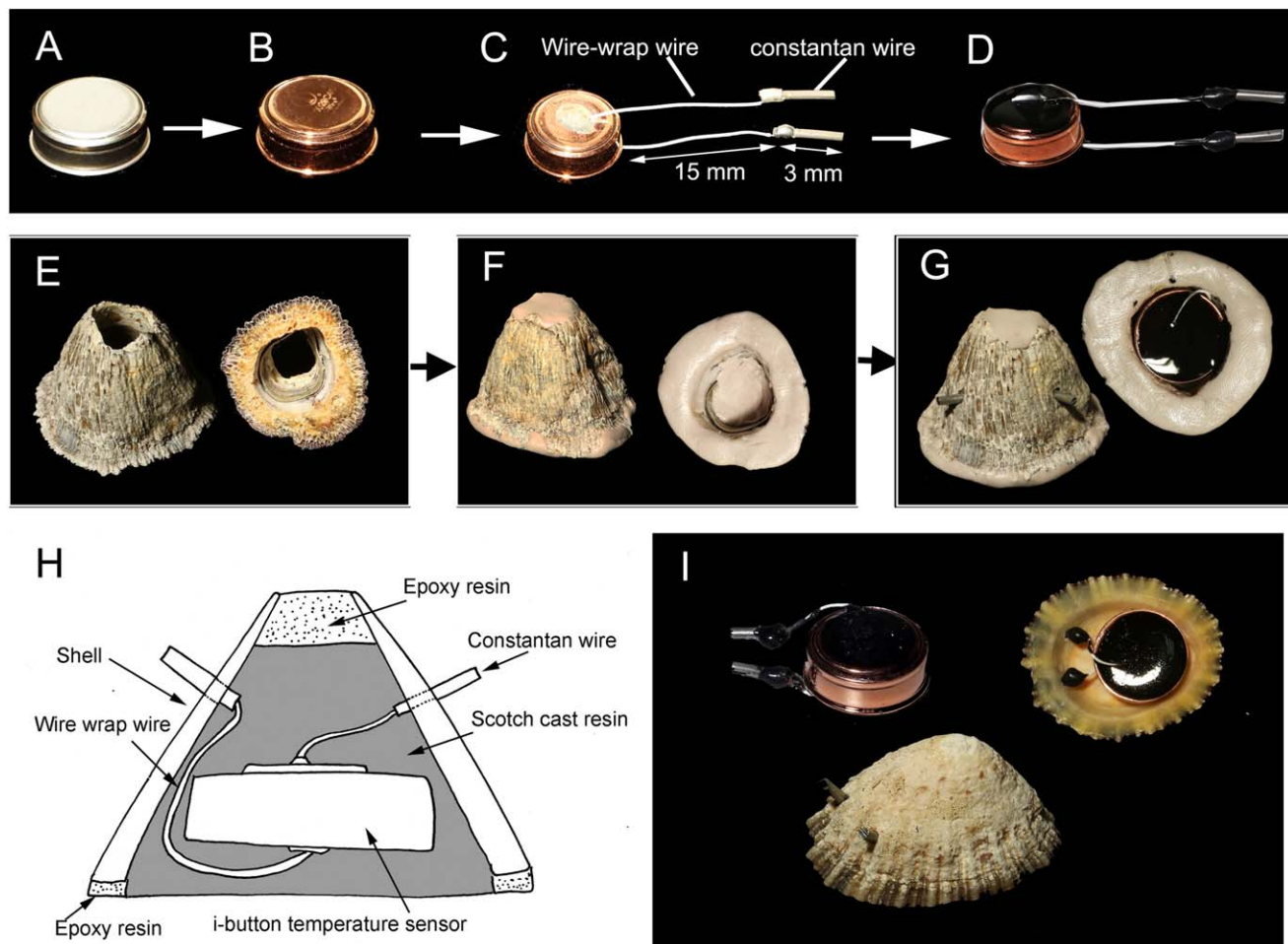
Fig. 1. Robobarnacle assembly sequence. (A) DS1922L iButton. (B) Copper-coated iButton. (C) Copper-coated iButton with soldered wire-wrap wires connected to two pieces of constantan wire. (D) Solder joints covered with a thin layer of Scotchcast 2130 Flame Retardant Compound to increase their strength. (E) Shell of *Tetraclita* used for robobarnacle construction. (F) Epoxy resin sealing the opercular opening and the basal tubes of the shell. (G) *Tetraclita* shell with the iButton inside and the constantan wires protruding through lateral holes, ready to be completely filled with Scotchcast. (H) Schematic anatomy of a robobarnacle. (I) Example of a robolimpet (in a *Patella vulgata* shell) built using the proposed new method of copper-coated iButtons.

miniaturized temperature sensors and biomimetic loggers, it has become possible to routinely obtain long-term temperature records closely matching the body temperatures of ectotherms. To have a good proxy of body temperature of ecototherms, the biomimetic loggers should have similar "body" sizes and thermal inertia to the studied animals. The design of such biomimetic loggers for intertidal systems is particularly challenging as the loggers must be waterproof, robust and also small, given the small body size of most intertidal animals (Fitzhenry et al. 2004).

In some earlier studies, Helmuth and Hofmann (2001) sealed Onset Corporation temperature loggers with silicone or epoxy plastic inside mussel shells to simulate in situ body temperature of live mussels and relate this with the expression levels of heat shock proteins 70 (HSP70). This approach revealed that the levels HSP70 in mussels was closely related to the tem-

perature recorded from the 'model mussels' rather than variations in the air or water temperatures (Helmuth and Hofmann 2001). Robert and Thompson (2003) introduced the technique for dehousing iButton sensors to produce smaller sized temperature loggers for biomimetic studies. Lima and Wethey (2009) adopted the techniques in Robert and Thompson (2003) and designed robolimpets—autonomous devices that mimic real limpets both visually and thermally—which have been successfully deployed along temperate Atlantic shores for long-term temperature monitoring (Seabra et al. 2011).

Robolimpets are built around a small circuit board (17.4 mm diameter) extracted from an iButton DS1922L logger and powered by a BR1225 (3V) lithium battery. The extraction of the circuit board involves using a rotary cutting tool to remove the stainless steel housing of the DS1922L iButton. The circuit board and battery are fitted into a limpet

Table 1. Hourly air temperature and solar irradiation obtained from the Keelung weather station of Central Weather Bureau, Taiwan (about 6 km from the study sites) during the sampling periods on 16th April 2013 and 17th April 2013. No rainfall occurred during the sampling periods

Time	Air temperature (°C)	Solar irradiation (MJ m^{-2} h^{-1})
16th Apr 2013		
9:00	25.3	2.07
10:00	24.3	2.82
11:00	24.1	3.16
12:00	23.4	2.40
13:00	23.2	2.15
17th Apr 2013		
11:00	28.0	2.53
12:00	29.2	2.45
13:00	29.5	1.43
14:00	29.3	1.20
15:00	29.1	1.16
16:00	28.2	0.93

shell and protected from the marine environment by a waterproofing resin. Two corrosion-proof constantan wires protruding from the limpet shell are used for downloading data and servicing the loggers on the shore (for more details *see* Lima and Wethey 2009).

The process of dehousing an iButton is not simple (Robert and Thompson 2003; Lima and Wethey 2009) and has several disadvantages. First, depending on the experience of the user, up to 25% of circuit boards are likely to be irreversibly damaged, which is a considerable loss since each iButton costs ∼ €35. Second, it is very easy to momentarily disconnect the power from the circuit board while dehousing the electronics, which causes the factory calibration to be lost. Calibration coefficients can be recalculated and used to correct readings *a posteriori* (Lima et al. 2011), but this is a time-consuming process. To address some of these concerns, in this study we describe an alternative and more efficient means of assembling biomimetic loggers that makes the whole process simpler, faster, and prevents calibration losses.

Materials and methods

Design of biomimetic data loggers

Robobarnacles

Barnacles of the genus *Tetraclita* are commonly found along tropical and subtropical intertidal ecosystems across the world and are, therefore, good models for monitoring the thermal stress of intertidal species in these regions (Chan et al. 2007). *Tetraclita* shells can reach 40 mm in external basal diameter, and those used for robobarnacle construction should have an external basal diameter of at least 30 mm in order to accommodate the DS1922L iButton (the same

model used for robolimpet production by Lima and Wethey 2009).

For this approach, the external case of the iButton was electroplated two times—first with nickel and then with copper—by a local electroplating company (at a cost of €1 per iButton; Fig. 1A,B). The plastic O-ring which makes the contact between the two halves of the iButton metal case was covered with a thin film of nail varnish to protect it during electroplating. Two wire-wrap wires (approximately 15 mm in length) were soldered to the two terminals of the copper-plated iButton case (Fig. 1C) and to two 1.6 mm diameter × 3 mm long constantan wires which are resistant to saltwater. The soldered junctions between the wire-wrap wires and both the copper-plated iButton case and the constantan wires were strengthened using a thin layer of Scotchcast 2130 (=2131, a replacement product for 2130) Flame Retardant Compound (3M, Fig. 1D). Both the basal part of the shell of *Tetraclita*, which is composed of numerous air spaces rather like a honeycomb, and the opercular opening (Fig. 1E) were sealed using household epoxy resin (3C's model 805 epoxy resin, Taiwan, Fig. 1F) to improve overall structural integrity. Each assembled logger was then inserted into a *Tetraclita* shell (Fig. 1G) while the constantan wires were allowed to protrude through two holes drilled on the side of the shell (Fig. 1G,H). Finally, the internal volume of the fully assembled robobarnacle was completely waterproofed with Scotchcast, allowing it to be deployed in the intertidal environment (Fig. 1H).

On the shore, robobarnacles were attached to the rock substrate using a thin layer of either 3C's model 805 epoxy resin (which hardens in 5 min after being thoroughly mixed) or xA-788 Z-Spar Splash Zone Compound (West Marine Ltd, California, U.S.A., which hardens in hours).

Robolimpets

To validate the methodology involving electroplating, we tested robolimpets constructed using the new methodology against robolimpets built according to Lima and Wethey (2009; hereafter referred to as the "old design"). For the new design, robolimpets were built using the same steps detailed above for *Tetraclita* robobarnacles, but loggers were instead inserted into shells of the limpet *Patella vulgata*. Both old and new design robolimpets were built using shells large enough to accommodate intact iButtons. Robolimpets were deployed on the shore using A-788 Z-Spar Splash Zone Compound following the methods of Seabra et al. (2011).

Field trials

Robobarnacles

Five robobarnacles were deployed adjacent (within 2 cm) to live *Tetraclita* barnacles of a similar diameter (± 3 mm) at Shen-Ao-Kang, NE Taiwan in April 2013 (for details of the study site, *see* Chen et al. 2013). A small hole (∼ 1 mm diameter) was carefully drilled through the shells of the live
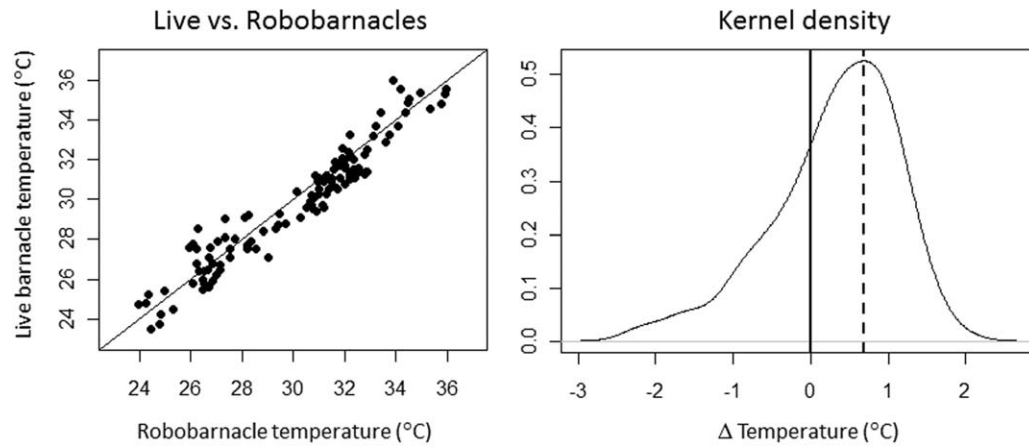
Fig. 2. (A) Scatterplot of robobarnacle vs. live barnacle temperatures for all pairs of robobarnacles and live barnacles. Diagonal line shows line of equality. (B) The kernel density of the temperature differences (i.e., temperature of robobarnacles – temperature of live barnacles). Note the differences peaked at 0.69°C, with the 95% confidence interval = (0.28°C, 0.91°C).

Table 2. Summary statistics of temperature differences between pairs of live barnacles (live) and robobarnacles (robo)

Pair	N	Mean temperature differences between robo and live (°C)	Root mean square deviation (°C)	Min temperature (°C) (live, robo)	Max temperature (°C) (live, robo)
1	21	−0.45	1.09	24.7, 24.0	29.2, 29.0
2	21	0.41	0.73	23.5, 24.5	28.0, 28.5
3	28	0.14	0.61	29.3, 29.5	35.6, 36.0
4	28	0.83	0.93	28.7, 29.4	35.3, 35.9
5	28	0.40	0.92	28.5, 29.3	36.0, 34.5
Total	126	0.15	0.86	—	—

barnacles, (~ 1 cm below the summit of the test), allowing a thin thermocouple to be inserted through the shell to measure the mantle temperature of the barnacles using a digital thermometer (CHY 502A, Taiwan, ± 0.1°C).

Temperatures were recorded from the live barnacles and logged by the robobarnacles at every 10 min from two pairs from 9:30 to 12:50 on 16th April, and from the remaining three pairs from 11:20 to 16:30 on 17th April (local time). On 16th April, the weather was sunny (solar irradiation ranged from 2.07 to 3.16 MJ m^{-2} h^{-1} and air temperature from 23°C to 25°C during the sampling period, Table 1), whereas on 17th April, the air temperature was higher, ranging from 28.0°C to 29.5°C (Table 1).
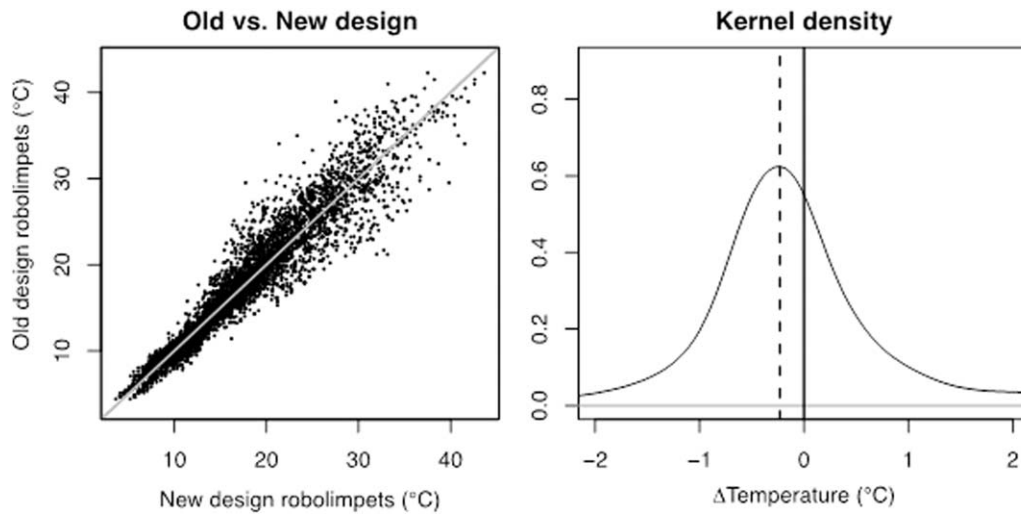
Robolimpets

Three pairs of new and old design robolimpets were deployed on south-facing rock faces at Moledo do Minho, NW Portugal (41.84°N, 8.87°W). Loggers from each pair were less than 10 cm apart, and all loggers were deployed in equivalent microhabitats (i.e., with similar shore heights, degrees of shading, exposure to wind and wave splash). Data were collected continuously between September 2013 and

November 2014, at a sampling interval of 60 min and a resolution of 0.5°C.

Statistical analysis

We quantified the temperature differences between live barnacles and adjacent robobarnacles based on the time series of the five pairs of comparisons. Specifically, we derived five time series of paired temperature differences between robobarnacles and live barnacles and estimated the kernel density of the temperature differences, pooling all of the time series data together. We used the normal kernel, with a recommended bandwidth (i.e., $0.9 \cdot \min(\hat{\sigma}, \text{IQR}/1.34) \cdot \hat{n} - 0.2$; Silverman 1986; Venables and Ripley 2002; where $\hat{\sigma}$ is the standard deviation of data points, IQR is the interquantile range, and n is data length) and weight (i.e., equal weight of all data points). We then derived the temperature difference associated with the peak of the kernel density and bootstrapped to estimate the 95% confidence intervals. The same procedure was used to evaluate differences between temperatures recorded by robolimpets built using the old and new designs. As temperatures collected by robobarnacles only comprised periods of aerial exposure, we discarded all data points recorded by robolimpets during immersion.

Fig. 3. (A) Scatterplot of old vs. new design robolimpet temperatures for all pairs. Diagonal line shows line of equality. (B) The kernel density of the temperature difference (i.e., temperature of old design robolimpets – temperature of new design robolimpets). Note the differences peaked at −0.23°C, with the 95% confidence interval = (−0.28°C, −0.21°C).

Table 3. Summary statistics of temperature differences between pairs of robolimpets assembled using the old and new designs.

Pair	N	Mean temperature differences between new vs. old design robolimpets (°C)	Root mean square deviation (°C)	Min temperature (°C) (old, new)	Max temperature (°C) (old, new)
1	3287	0.16	1.60	6.6, 5.2	36.7, 38.7
2	2249	0.43	1.10	4.4, 3.6	42.4, 43.6
3	4082	−0.24	1.58	4.5, 5.2	42.1, 38.2
Total	9618	0.05	1.49	—	—

Results and discussion

Temperature measurements on 16th and 17th April were generally consistent between live barnacles and the robobarnacles, as the scatterplots for these pairs approximately followed an isoline (Fig. 2A). The kernel density of the temperature difference (i.e., temperature of robobarnacles – temperature of live barnacles) peaked at 0.69°C with the 95% confidence interval = (0.28°C, 0.91°C) (Fig. 2B). Also, the minimum as well as the maximum temperature readings were similar between the live vs. robobarnacles of each pair (Table 2).

Temperatures obtained from robobarnacles were, therefore, very similar to those experienced by live barnacles, suggesting that the proposed design can be used to estimate body temperatures of individuals of *Tetraclita* species. In some cases (pairs 2, 4, and 5), robobarnacle temperatures were slightly (0.4–0.8°C), but consistently, higher than those registered in live animals (Table 2). Such differences may be a result of very small scale differences in location, such as variation in the specific heat capacity of Scotchcast as

compared to the living animals, or more likely the behaviour of live barnacles under thermal stress, when they slightly open their opercular valves, allowing some of the mantle water to evaporate, thus reducing their body temperatures via evaporative cooling (Chan et al. 2006). Robobarnacles lack this ability, which may explain their slightly higher temperatures during the hottest periods of the day. Still, overall temperature deviations between the robobarnacles and live barnacles is comparable to the values reported for robolimpets using Lima and Wethey's design (1.06°C in Lima and Wethey 2009 and less than 1.2°C in Lathlean et al. 2015). In addition, temperatures recorded using robolimpets built using the electroplating method very closely matched those recorded by the old design robolimpets (bias of −0.23°C with 95% confidence interval = (−0.28°C, −0.21°C); Fig. 3, Table 3). This further supports the notion that the electroplating methodology is valid and consistent with the thoroughly tested and field-proven original design by Lima and Wethey (2009).

Compared to the present method, Lima and Wethey's (2009) design is able to mimic smaller specimens and has

Table 4. Comparison between the design of biomimetic loggers in Lima and Wethey (2009) and the present study.

	Lima and Wethey (2009)	Present study
Construction	Need to extract electronics from the iButton steel case.	No need to open the iButton's stainless steel case.
	No need for copper electroplating. Relatively high loss rate during assemblage.	Copper electroplating necessary. Very low loss rate.
Appropriate shell size	Small to large size	Large sized animals only
Recalibration	Need to recalibrate if the battery disconnects from the circuit during assemblage.	Recalibration is not necessary.
Battery	Can be changed to a higher capacity battery.	Battery changing not possible.
Chance of retrieving data from broken loggers	Very low. Without the original casing the likelihood of water reaching the circuit on the breaking of the outer shell is greatly increased, typically leading to irreversible damage due to corrosion.	Very high. By not removing the original casing loggers have two layers of waterproofing protection and, frequently, intact iButtons can be recovered from broken loggers, allowing for data recovery.
Precision (Root mean square deviation)	Average 1.06°C ± 0.39°C (SD) deviation from live limpets.	Average 0.86°C ± 0.19°C (SD) deviation from live barnacles.

the additional benefit of allowing the original iButton battery to be replaced by a larger-capacity battery. Conversely, the major advantage of our new design is the ability to save an appreciable amount of time by avoiding iButton disassembly (and the risk of damage, and hence increased costs) and the need to recalibrate loggers if power supply is lost during manufacture (*see* Table 4). The robobarnacles deployed for this project continued logging data for 1 year while surviving several typhoons which impacted the deployment site. During this time, ~ 15% of the robobarnacles deployed had their tests damaged or broken by wave or loose boulder impact, and in some cases lost their external constantan wires. Despite the external damage, in all cases it was possible to extract the iButtons from the waterproofing resin and recover the logged data in the laboratory, and to recycle the recovered iButtons for use in new robobarnacles. Such flexibility is not possible in the design proposed by Lima and Wethey (2009) as the integrity of the iButton is not maintained during logger production, making it nearly impossible to successfully extract the circuit board from the resin once it is enclosed in the shell. Furthermore, the revised methodology described here can be easily modified to produce mimics of large individuals (> 25 mm shell length) of limpets such as *Patella vulgata*, *P. ulyssiponensis*, *Cellana grata*, *C. testudinatus* and *C. nigrolineatus* and mussels such as *Mytilus* spp. (*see* Fig. 1I for an example of a robolimpet produced following the copper electroplating methodology).

Overall, this new design is less expensive, easier to assemble, more precise, and markedly more robust, suggesting it should be used preferentially whenever the studied animal is of sufficient size. The reduction in cost and the increased likelihood of obtaining long datasets without gaps represents a significant step forward for researchers designing and maintaining continental-scale networks of temperature sensors.

Conclusion

In this study, we described a simplified and reliable process to assemble biomimetic loggers, and use it to build robobarnacles (mimicking tropical and subtropical barnacles of the genus *Tetraclita*) and robolimpets. Our approach makes the manufacturing process simpler, faster, and prevents calibration loss, but is only suitable for species large enough to accommodate a complete iButton.

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