Original Article

Coping with social stress: heart rate responses to agonistic interactions in king penguins

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In colonial breeders, agonistic interactions between conspecifics are frequent and may have significant physiological implications. Physiological responses (e.g., increased heart rate) to such social stressors may be determined by the potential costs of agonistic interactions, such as personal injury or risk of breeding failure, and by the motivation of the individuals concerned. The latter may vary according to individuals' reproductive status or willingness to engage in agonistic interactions. In this study, we investigated heart rate responses to aggressive interactions in a breeding colony of king penguins *Aptenodytes patagonicus*. From heart rate (HR) and behavior recorded in 20 adults at various stages of the breeding season, we investigated how king penguins reacted to aggressive neighbors. A total of 589 agonistic interactions, 223 in which birds were actors and 366 in which birds remained bystanders (i.e., witnesses that were not involved in interactions), were characterized. We found that HR increased during agonistic interactions, both in actors and bystanders. The intensity (threat displays or physical attacks), duration, and rate of aggressive events (number of threats/blows per unit time) of an interaction significantly influenced the HR response in actors. For bystanders, however, only the duration of interactions seemed to matter. Our results also suggest a role for individual motivation, as initiators of agonistic interactions displayed higher HR increases than responders, and as increases were not constant throughout the reproductive season. We conclude that individual risk assessment and motivation modulate physiological responses to social stressors in group-living animals. *Key words:* aggressive behavior, colonial breeding, context assessment, heart rate modulation, motivation, stress. *[Behav Ecol]*

INTRODUCTION

Reaping individual fitness benefits from clustering with conspecifics has paved the road for the evolution of group-living and animal sociality (Alexander 1974; Wilson 1975; Baglione et al. 2002; Viblanc et al. 2010; Dobson et al. 2012). However, sociality comes with costs. Individual requirements must be compromised with those of other group members for animal groups to retain their integrity (West-Eberhard 1979; Armitage and Schwartz 2000; Sueur et al. 2010). The social environment hence has strong effects on individual behavior (Boissy and Le Neindre 1997) and physiology (Cacioppo 1994), including hormone secretion (Creel 2001; Oliveira et al. 2001; Goymann and Wingfield 2004), neurotransmitter secretion (Edwards and Kravitz 1997), immune function (Bartolomucci 2007), and regulation of the autonomous nervous system, as reflected by changes in heart rate (HR) (Berntson and Boysen 1989; Aureli et al. 1999; Wascher et al. 2008a).

Evidence that social interactions may strongly affect stress responses in free-living animals is accumulating (Oliveira et al. 2001; Wascher et al. 2008a,b; Wascher et al. 2009; Mouterde et al. 2012, see Cacioppo 1994, Kemeny 2003), and how stress responses may be shaped depending on social context and associated risk (Wascher et al. 2009; Mouterde et al. 2012) is of central interest to our understanding of the *pros* and *cons* of group-living. For instance, in a series of studies considering social interactions in greylag geese (*Anser anser*), Wascher and colleagues (2008a,b, 2009) recently showed that the individual's HR response to stress was subject to both the nature and intensity of the social context, as well as to the identity of those involved. The authors argued that such differences in physiological responses might reflect differences in individuals' motivation depending on the social context (Wascher et al. 2009).

The case of aggressiveness and agonistic interactions is of particular relevance for social contexts. Intraspecific competition is ubiquitous and agonistic interactions are commonly performed when individuals defend undividable resources such as territories, food, or mates. The fitness benefits of defending a resource must outweigh the costs associated with its defense for agonistic behaviors to evolve and persist (Maynard-Smith 1982; Maynard-Smith et al. 1988). The costs of agonistic interactions may come in many forms, including physical injury, time and energy investment, and physiological costs. Of particular interest is the finding that such physiological effects may also be experienced by bystander individuals: social group members who are only witnesses to such interactions (Oliveira et al. 2001; Wascher et al. 2008b). There is thus little doubt that specific social contexts, such as agonistic interactions differing in risk for instance, may elicit varying physiological responses in different individuals, and how important these responses may be in terms of physiological investment merits further consideration.

In this study, we investigated HR responses of free-living, colonial king penguins (*Aptenodytes patagonicus*) to agonistic interactions, which were bouts of either threat displays or physical attacks with body contact. HR is a highly sensitive physiological parameter that may be used to investigate individuals' reactivity to the social environment (Nakagawa et al. 2001; Wascher et al. 2008a) and responsiveness to

stress ([Nephew et al. 2003](#); [De Villiers et al. 2006](#)). In king penguins, reproductive birds crowd on shores where they vigorously defend a small territory (approximately 0.5 m²), exhibiting a high rate of agonistic interactions while incubating their only egg or brooding their young chick in their brood pouch. Indeed, 14% of daily time-budget is devoted to territory defense ([Viera et al. 2011](#)) and a mean of 100 interactions per bird per hour is observed at this time ([Côté 2000](#)). When incubating the egg or brooding the young chick on their feet, breeding parents move little, remaining on their small breeding territory, surrounded by neighboring breeders. The action-radius of breeding birds for territorial defense is thus limited to their closest neighbors, on average 5 individuals within a 50-cm distance, and passing birds within this radius (i.e., birds transiting through the colony to access or leave their breeding territory). For those reasons, king penguins present an interesting model for evaluating changes in social stress responses in a strong context of aggressiveness, an attempt which has rarely been achieved in the wild (see [Wascher et al. 2009](#)).

After characterizing agonistic interactions in king penguins (see below), we examined whether focal king penguins elicited noticeable HR responses during these interactions either when actively taking part in (actors) or remaining witness to (bystanders) the interactions. We expected that HR responses would occur during conflicts and predicted that conflicts would also elicit HR responses in bystanders watching aggressive neighbors, as they may be concerned by redirected aggression which is common in king penguin (personal observations), bystanders often ending up being involved in neighboring conflicts. Further, we investigated whether HR responses were influenced by the intensity of agonistic interactions (i.e., threat displays or physical blows). We also considered whether the characteristics of agonistic interactions such as duration, number of individuals involved, and rate of aggressive events (number of threats/blows exchanged per unit time) affected HR responses, as all of those factors are expected to influence the risk (e.g., injury, egg/chick-loss) of specific interactions. Lastly, we considered the possibility that the motivation of individual birds, depending on their role as initiators versus receivers in agonistic interactions, or depending on timing in the breeding season and motivation to breed, could modulate the HR response.

METHODS

Study population and ethical considerations

This study was conducted throughout 2 consecutive breeding seasons between 2008 and 2010 (from late December to late March) on Possession Island, Crozet Archipelago (46°25'S, 51°45'E). King penguins were monitored in a subcolony of approximately 3500–4000 pairs in the colony of "La Baie du Marin," which is host to some 23 000 pairs of king penguins. Twenty-five pairs (50 birds) were marked using a nonpermanent animal dye (Porcimark®, Kruuse, Langeskov, Denmark) and flipper-banded using semi-rigid P.V.C Darvic bands (25.8mm wide, 1.9mm thick, 7.4g) for field identification during HR recording and behavioral observations. Both males and females were banded at the start of their first incubation shift. Males were banded shortly after the female laid the egg and departed for her foraging trip at sea, whereas females were banded on their return, when relieving their incubating partner some 15 days later. Birds were followed daily throughout their successive incubation and brooding shifts, in order to determine the exact breeding stage of focal birds during HR recording and behavioral observations (see below). In this study, although birds were of unknown age, the average age at first reproduction in king penguin is 6 years ([Weimerskirch et al. 1992](#)) and only a marginal fraction of juveniles attempt to breed earlier, at 3 or 4 years of age ([Weimerskirch et al. 1992](#); [Saraux et al. 2011](#)a). All procedures described in the following sections and employed during the fieldwork were approved by the Ethical Committee of the French Polar Institute (Institut Polaire Français Paul Emile Victor, IPEV) and comply with current French laws. Authorizations to enter the breeding colony and handle a limited number of birds were delivered by the French Committee for Polar Environments and by the Terres Australes et Antarctiques Françaises. Flipper bands were removed at the end of the study because of their known long-term detrimental effect on penguins ([Gauthier-Clerc et al. 2004](#); [Saraux et al. 2011](#)b).

Heart rate recording

We used externally mounted HR-loggers (Polar® model RS800, Polar Electro Oy, Kempele, Finland) to monitor the HR response of king penguin breeding in their natural colony to the agonistic interactions of conspecifics. Loggers were specially adapted for suitable use on king penguins and accurately recorded HR within the HR range of free-living birds (see [Groscolas et al. 2010](#) for details). The HR-logger unit was set to store the sampled data for up to 31.5h, sampling at a rate of 1 data point per second, which was appropriate considering the short duration of the considered interactions (around 9 s, see Results). Electrodes were composed of 2 stainless-steel wires attached to gold-plated safety pins, which were placed subcutaneously on the dorsal region of the animal. The whole apparatus was attached using adhesive tape (Tesa®) to the dorsal feathers of the birds and weighed less than 1% of total body mass. Loggers and electrodes were out of the reach of animals and did not interfere with their usual behavior, as equipped individuals soon resumed normal activity after handling (e.g., preening, stretching, sleeping, and fighting) and could not be distinguished from other individuals in behavioral patterns during the subsequent day ([Viblanc et al. 2011](#); [Viera et al. 2011](#)). After equipment, birds were left to recover overnight and video recording was resumed the next day.

Behavioral observations

When incubating and brooding, male and female king penguin alternate shifts ashore for tending the egg or chick and periods at sea for foraging ([Stonehouse 1960](#); [Weimerskirch et al. 1992](#)). Incubation and brooding shift duration average 15 and 8 days, respectively, the total duration of incubation being 53 days. A total of 20 birds (12 males and 8 females), at various shifts of incubation or chick-brooding (see [Table 1](#)), were equipped with a HR-logger and their behavior was monitored simultaneously to HR for a period of 6h per bird. All birds were monitored close to the start of their respective breeding shift (usually some 3 days after they started incubating or brooding), to avoid potential confounding effects, such as potential increased stress for some birds due to delayed return of a partner. In addition, apart from males in shift 1 of incubation, all birds had thus been fasting for a similar amount of time. In 2008–2009, we monitored a total of 11 incubating birds (7 males and 4 females), of which 2 males were monitored both during their first and second incubation shift (incubation shifts 1 and 3, see [Table 1](#)). Chick-brooding birds (5 males and 4 females) were monitored for a single brooding shift in 2009–2010. Bird behavior was recorded using a Sony DCR HC53 Handycam that was set on a tripod at a maximal distance of 10 m to the focal individual. The camera was centered on the focal individual

Table 1

Characteristics of the 20 focal penguins (12 males and 8 females) monitored in the study

	Males													
#	1	2	3	3	4	4	5	6	7	8	9	10	11	12
Shift(s)	S1	S1	S1	S3	S1	S3	S3	S3	S3	S5	S5	S5	S5	S5
n interactions	15	27	37	34	22	18	33	35	30	17	25	28	27	26
Baseline HR	41.7 ± 2.0	42.5 ± 1.2	54.5 ± 0.7	56.9 ± 0.8	72.9 ± 2.4	46.5 ± 0.9	63.6 ± 0.9	75.0 ± 1.7	51.1 ± 0.8	80.5 ± 2.4	72.7 ± 0.9	75.3 ± 1.6	98.7 ± 1.4	64.4 ± 1.2

	Females							
#	1	2	3	4	5	6	7	8
Shift(s)	S2	S2	S2	S2	S6	S6	S6	S6
n interactions	20	41	24	29	25	18	28	30
Baseline HR	60.7 ± 1.5	83.0 ± 1.6	76.3 ± 1.3	73.1 ± 0.8	69.4 ± 1.3	63.5 ± 2.1	72.9 ± 1.5	54.8 ± 0.6

The breeding shifts during which birds were monitored, the number of agonistic interactions analyzed per bird, and the average baseline HR before agonistic interactions are given. Shifts S1–S3 are for incubating birds, shifts S5 and S6 for chick-brooding birds.

and the picture zoomed in to consider a circle delimited by the focal individual in the center and neighbors within a 3-m radius. In order to time-match behavioral observations with changes in HR, penguin HR-loggers and the camera's clock were synchronized to the nearest second. From the 120 h of behavior recording, we selected a total of $n = 589$ agonistic interactions. A selected interaction was always preceded and succeeded by a period during which the focal bird and its neighbors were resting, HR of focal birds being stabilized at baseline levels. The agonistic interaction was considered to start when the focal individual or at least 1 bird in its immediate surrounding (neighbors within approximately 1.5-m radius circle) engaged in aggressive behavior (threat displays or physical blows, Côté 2000; Viera et al. 2011), and to stop when all birds within this radius (i.e., approximately 25 birds) resumed a vigilant or resting position. We only considered interactions for birds that were holding a breeding territory (not passing individuals), and did not consider neighbors outside this radius because preliminary behavioral observations showed that the focal bird was not reactive to behavior of birds located at a greater distance. Interactions were also selected so that increases in HR could be attributed to aggressive behavior alone. Altogether, this involved discarding a large number of agonistic interactions where birds did not appear to be behaviorally resting before or after the interaction, or where HR increases might have been confounded by other behaviors during the interaction (e.g., preening, singing, egg or chick-cares). High-intensity interactions ($n = 366$; range = 8–48 per bird) consisted of attacks with physical contact (beak or flipper blows) and some threat displays, whereas low-intensity interactions ($n = 223$; range = 5–23 per bird) consisted solely of threat displays (beak pointing and gaping, i.e., vocalizing beak open toward a conspecific). In this study, we refer to high- and low-intensity interactions as "blows" and "threats," respectively.

Data analysis

First, we investigated whether breeding king penguins showed noticeable HR responses during agonistic interactions and if so, whether this response was affected by individual involvement in (actors vs. bystanders), or by the intensity (threats vs. blows) of, interactions. Whereas actors were actively involved (either initiating or being the target of agonistic interactions), bystanders remained still (no physical activity) and vigilant when observing neighboring birds interacting within a distance of 1.5 m. We compared the HR response of actors and bystanders by calculating mean and maximum

HR increase, as well as HR excess during agonistic interactions. HR increases were calculated as the difference between the momentary baseline HR value 2–3 s before the interaction and either the mean HR value during the interaction (mean HR increase, until HR reached baseline levels again) or the instantaneous maximum HR value reached during the interaction (maximum increase). As mean and maximum HR increases were highly correlated (Spearman's rho test, $r = 0.95$, $P < 0.001$, $n = 589$) and yielded similar results, we chose to present only maximum HR increase in our results. HR excess was calculated as the mean HR increase over baseline HR (in beats per minute, bpm) times the duration of that increase (in minutes). HR excess corresponded to the area under the curve of the HR increase during agonistic interactions, and represented the number of heart beats produced in excess of what would have been produced had HR been maintained at baseline levels. As no significant sex difference was noted in HR excess and maximum HR increase during agonistic interactions (generalized linear mixed models [GLMMs]: $z = 1.9$ and 1.8, $P = 0.09$ and 0.10, $n = 589$, 20 birds, respectively), both sexes were pooled in the analysis not considering specific incubation or brooding shifts. Independent variables regarding the nature of agonistic interactions included: 1) the intensity of considered interactions (threats or blows), 2) the number of individuals involved within a 1.5-m radius circle around the focal bird, 3) the duration of aggressive encounters, and 4) the rate of aggressive events within an interaction (i.e., the number of blows or threats exchanged during the interaction divided by the duration of the interaction). In addition, for actors, we distinguished the rate of aggressive events occurring between the focal individual and its neighbors ($rate_1$) and the rate of aggressive events occurring solely between neighbors ($rate_2$), as the former is associated with a greater risk of injury for the focal animal than the latter.

Furthermore, we investigated whether changes in HR responses were observable throughout the course of the breeding season. As breeding shifts are sex-specific in king penguin, we tested for an effect of breeding advancement by comparing sex-specific shifts together.

Statistics

Statistical analyses were performed using the R v.2.10.1 (http://www.r-project.org/) software. We used GLMMs including bird identity as a random factor to account for interindividual variation in HR responses and repeated measurements on the same individual. The potential effect

of the interactions' characteristics (i.e., independent variables: duration, number of individuals involved, and rate of aggressive events within an interaction) on HR responses (i.e., dependent variables: HR excess or maximum HR increase) was investigated separately in actors ($GLMM_{act}$) and bystanders ($GLMM_{bys}$). As HR responses were shown to differ between blows and threats, we included intensity (i.e., threats vs. blows) as a cofactor in the models and considered the statistical interactions of intensity with all other independent variables (see above). We thus first performed full models including all variables and interactions. We then excluded least-significant terms (starting with interactions) in a stepwise procedure, using Akaike's Information Criterion (AIC) to select the best models. Specifically, removing the least-significant term, we compared the model's AIC with the next best fit, and retained it as the best model only if ∆AIC was ≥2 (Burnham and Anderson 2002). We kept excluding nonsignificant terms as long as AIC decreased and ∆AIC was ≥2. Whenever ∆AIC < 2 between 2 models, we retained the most parsimonious one (i.e., the one with the less terms) as the best fit. As residuals were nonnormal, models were fitted with negative-binomial distribution using the "glmmADMB" package in R (Skaug et al. 2011), which was appropriate considering the distribution of our dependent variables, and working with over-dispersed data (O'Hara and Kotze 2010). Significant effects are reported for $P < 0.05$. Results are given as means ± standard error (SE).

RESULTS

Characteristics of agonistic interactions

Overall, the agonistic interactions observed lasted for 8.9 ± 0.2 s (range = 1–34 s, $n = 589$). Agonistic interactions with blows lasted 26% longer than interactions with threat displays, that is, 9.7 ± 0.3 s ($n = 366$) versus 7.7 ± 0.3 s ($n = 223$), respectively (GLMM: $z = -4.9$, $P < 0.001$, $n = 589$, 20 birds). On average, interactions with blows involved a higher number of participants than did interactions composed solely of threat displays, that is, 2.5 ± 0.1 birds (range = 1–12, $n = 366$) versus 2.0 ± 0.1 birds (range = 1–6, $n = 223$), respectively (GLMM; $z = -3.01$, $P = 0.003$, $n = 589$, 20 birds). The average rate of aggressive events within an interaction was more than double that for interactions with blows (0.9 ± 0.03 events/s) than for interactions with threats (0.4 ± 0.02 events/s) (GLMM: $z = -5.69$, $P < 0.001$, $n = 589$, 20 birds).

HR responses during agonistic interactions

On the involvement of individual birds and the nature of agonistic interactions

Baseline resting HR averaged 64 ± 1 beat per min (bpm) in males ($n = 12$) and 70 ± 1 bpm in females ($n = 8$). Overall and considering actors and bystanders together, we found penguins to elicit strong HR increases above baseline resting values in response to agonistic interactions. Changes in HR were instantaneous, occurring on average 0.2 ± 0.1 s after the beginning of the interaction, and lasting for an average duration of 10.4 ± 0.2 s, comparable with that of the interaction. During blows, maximum HR increase and HR excess were 17.6 ± 0.8 bpm and 2.0 ± 0.1 bpm, respectively, whereas during threats these were 12.9 ± 0.8 bpm and 1.2 ± 0.1 bpm, respectively (Figure 1a,1b). Maximum HR increases were significantly different from zero both during blows and threats, estimated intercepts being highly significant in models where only the random effect was included (GLMMs: $z = 35.0$ and 37.8, $P < 0.001$, $n = 589$, 20

birds, for blows and threats, respectively). HR excess, however, was significantly different from zero only during blows (GLMMs: $z = 5.12$ and 1.07, $P < 0.001$ and $P = 0.29$, $n = 589$, 20 birds, for blows and threats, respectively). Considering actors and bystander separately, the HR response of actors was greater than that of bystanders: HR excess was on average 65% higher (i.e., 2.3 ± 0.2 beats vs. 0.8 ± 0.05 beats, Figure 1a; GLMM: $z = -11.1$, $P < 0.001$, $n = 589$, 20 birds) and maximum HR increase at least twice as great (20.1 ± 0.9 bpm vs. 10.0 ± 0.4 bpm, Figure 1b; GLMM: $z = -11.3$, $P < 0.001$, $n = 589$, 20 birds) in actors than it was in bystanders. HR excess was significantly different from zero both in actors ($P < 0.001$) and bystanders ($P = 0.0014$), as was the maximum HR increase ($P < 0.001$, for both actors and bystanders). Considering the intensity of agonistic interactions (viz., blows or threats), whereas actors appeared more responsive to blows (displaying higher HR responses during such interactions), this was not the case for bystanders (Figure 1a,1b).

On individual motivations during agonistic interactions
Considering only the case of actors, we investigated whether noticeable differences were observable when birds either initiated or responded to threats or blows. Overall and whatever the intensity of the interaction, when included in a GLMM, HR responses were generally higher when birds were initiators rather than responders (GLMM: $z = -3.31$, $P < 0.001$, $n = 341$, 20 birds). When considering threats and blows separately, we found that whereas actors showed higher HR responses when initiating rather than responding to blows (GLMM: $z = -3.22$, $P < 0.001$, $n = 212$, 20 birds; Figure 2a), the difference was not significant concerning threats (GLMM: $z = -0.34$, $P = 0.73$, $n = 129$, 20 birds; Figure 2b).

On the influence of the agonistic context
For actors, HR responses increased with the duration of the interactions and the rate of aggressive events (both $rate_1$ and $rate_2$) that occurred during an agonistic interaction. However, the number of individuals involved in the agonistic interaction had no significant effect on HR responses (Table 2). The best fitting model actually included a significant statistical interaction between intensity (threats or blows) and the duration of the agonistic interactions. Indeed, the effect of the duration of the interaction on HR responses was more than
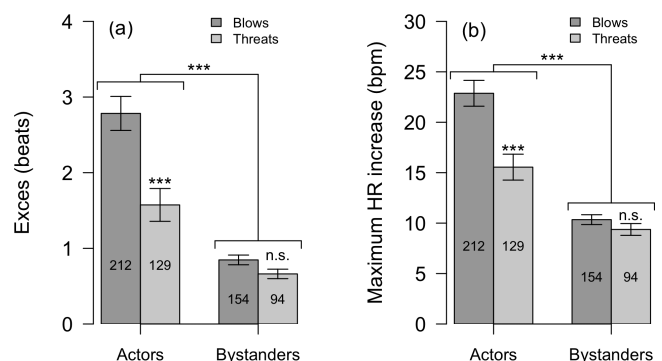
Figure 1
Heart rate (HR) responses to agonistic interactions (actors or bystanders, physical blows or threat displays) in breeding king penguins. (a) HR excess caused by the agonistic interaction (in beats). (b) Maximum HR increase above pre- and postinteraction baseline values (in bpm). Values are given as means ± SE. Number of agonistic interactions is figured in the bars. ***$P < 0.001$, n.s., nonsignificant.

Table 2

Model selection to explain HR excess variability depending on the characteristics of agonistic interactions in king penguin actors

Actors (GLMMs$_{act}$)

N°	Dependent variable	Characteristics of the agonistic interaction	Statistical interactions	AIC	ΔAIC	k	n	N (birds)
1	HR excess	Dur + N_i + rate$_1$ + rate$_2$ + intensity	Intensity * dur, intensity * N_i, intensity * rate$_1$, intensity * rate$_2$	1205.7	4.7	12		
2	HR excess	Dur + N_i + rate$_1$ + rate$_2$ + intensity	Intensity * dur, intensity * rate$_1$, intensity * rate$_2$	1204.5	3.5	11		
3	HR excess	Dur + rate$_1$ + rate$_2$ + intensity	Intensity * dur, intensity * rate$_1$, intensity * rate$_2$	1203.2	2.1	10	341	20
4	HR excess	Dur + rate$_1$ + rate$_2$ + intensity	Intensity * dur, intensity * rate$_1$	1201.3	0.2	9		
5	**HR excess**	**Dur + rate$_1$ + rate$_2$ + intensity**	**Intensity * dur**	**1201.1**	**0**	**8**		

Generalized mixed models (GLMMs) were fitted with a negative-binomial distribution. Bird identity was included as a random variable in all models. Independent variables include the duration (dur) of the agonistic interaction, the number of individuals (N_i) involved in the interaction, the rate of aggressive events (number of blows/threats per unit time) occurring between the focal individual and its neighbors (rate$_1$), and solely between neighbors (rate$_2$). The intensity of the interaction (treats vs. blows) was included as a cofactor in the models. Nonsignificant terms were removed sequentially (starting with interactions) from the full model. The best model retained was the one with the lowest AIC and the fewest parameters (most conservative model), and is indicated in bold. ΔAIC is the difference of AIC compared with the best model, k is the number of parameters in the model, n is the number of agonistic interactions, N the number of birds. Fitting GLMMs to maximum HR increase yielded similar results.
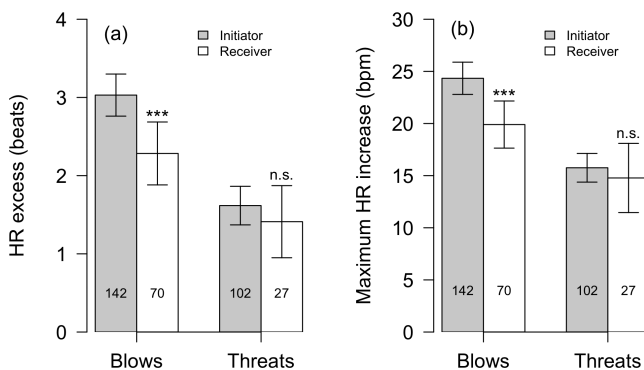

Figure 2
Heart rate (HR) responses to agonistic interactions (actors, physical blows or threat displays) in breeding king penguins, depending on whether the focal individual was the initiator or the receiver of the interaction. HR excess and maximum HR increase as in Figure 1. Values are given as means ± SE. Number of agonistic interactions is figured in the bars. ***$P < 0.001$, n.s., nonsignificant.

double during threats than during blows (i.e., higher parameter estimates for threat displays than for blows, 0.13 ± 0.02 vs. 0.06 ± 0.01 and 0.08 ± 0.01 vs. 0.03 ± 0.01 for HR excess and maximum HR increase, respectively).

For bystanders, only the duration of agonistic interactions had a significant positive influence on their HR response (Table 3). HR responses did not differ according to the intensity of the interaction (threats or blows), rate$_2$ (bystanders being witnesses, only rate$_2$ may be calculated), or the number of participants in the interaction. In addition, none of the statistical interactions including the intensity of the interaction were significant (all $P > 0.98$).

Variations throughout the breeding season
Variations in HR responses of actors and bystanders during the breeding season are reported in Figure 3. Overall, considering actors and bystanders together and whatever the intensity of the agonistic interactions, breeding shift

(considered as a continuous variable) did not affect HR responses for males ($P = 0.42$ and 0.13, for HR excess and maximum HR increase, respectively) or females ($P = 0.48$ and 0.80, for HR excess and maximum HR increase, respectively). As incubating and brooding birds were sampled in different years, we could not test for a potential effect of breeding status (incubating vs. brooding), as this may have been confounded with a potential seasonal effect. However, for males we were able to test whether HR responses differed between the first and the third incubation shift, as those were monitored during the same year. We found that incubating actors tended to elicit higher HR responses when exchanging physical blows during the third breeding shift (close to egg hatching) than during the first breeding shift (GLMMs: $P = 0.04$ and $P = 0.03$, $n = 158$, 7 birds, for HR excess and maximum HR increase, respectively). For instance, maximum HR increase was 60% more for breeding shift 3 (27.2 ± 2.6 bpm) than for breeding shift 1 (16.9 ± 2.0 bpm). This difference, however, was not apparent concerning threats, nor did we find any significant difference in bystanders (all $P > 0.6$).

DISCUSSION

In this study, we investigated the effects of agonistic interactions (social stressors) on the HR of colonial seabirds breeding in a highly aggressive social environment. We show that HR increases were elicited both by individuals directly concerned by agonistic interactions and also by individuals standing nearby. Agonistic interactions provoked maximum increases in HR as great as 30% of resting values in actors and 16% in bystanders, that is, half that of actors. In actors, the changes observed in HR during agonistic interactions are likely to reflect increased energy expenditure associated with physical activity (Viera et al. 2011). Indeed, during fights, the necessity to increase fuel and oxygen supply to muscles will cause HR to increase at least to some extent (Viera et al. 2011). Thus, it is not surprising to find that during agonistic interactions actors displayed greater HR increases than bystanders, and that HR increases were greater during blows than during threats; the latter being associated with

Table 3

Model selection to explain HR excess variability depending on the characteristics of agonistic interactions in king penguin bystanders

Bystanders (GLMMs$_{bys}$)

N°	Dependent variable	Characteristics of the agonistic interaction	Statistical interactions	AIC	ΔAIC	k	n	N (birds)
1	HR excess	Dur + N_i + rate$_2$ + intensity	Intensity * dur, intensity * N_i, intensity * rate$_2$	521.8	10.2	10		
2	HR excess	Dur + N_i + rate$_2$ + intensity	Intensity * dur, intensity * N_i	519.8	8.2	9		
3	HR excess	Dur + N_i + intensity	Intensity * dur, intensity * N_i	517.8	6.2	8	248	20
4	HR excess	Dur + N_i + intensity	Intensity * dur	515.8	4.2	7		
5	HR excess	Dur + N_i + intensity		513.8	2.2	6		
6	HR excess	Dur + N_i		512.7	1.1	5		
7	**HR excess**	**Dur**		**511.6**	**0**	**4**		

Generalized mixed models (GLMMs) were fitted with a negative-binomial distribution. Bird identity was included as a random variable in all models. Independent variables include the duration (dur) of the agonistic interaction, the number of individuals (N_i) involved in the interaction, and the rate of aggressive events (number of blows/threats per unit time) occurring between neighbors (rate$_2$). The intensity of the interaction (treats vs. blows) was included as a cofactor in the models. Nonsignificant terms were removed sequentially (starting with interactions) from the full model. The best model retained was the one with the lowest AIC and the fewest parameters (most conservative model), and is indicated in bold. ΔAIC is the difference of AIC compared with the best model, k is the number of parameters in the model, n is the number of agonistic interactions, N the number of birds. Fitting GLMMs to maximum HR increase yielded similar results.
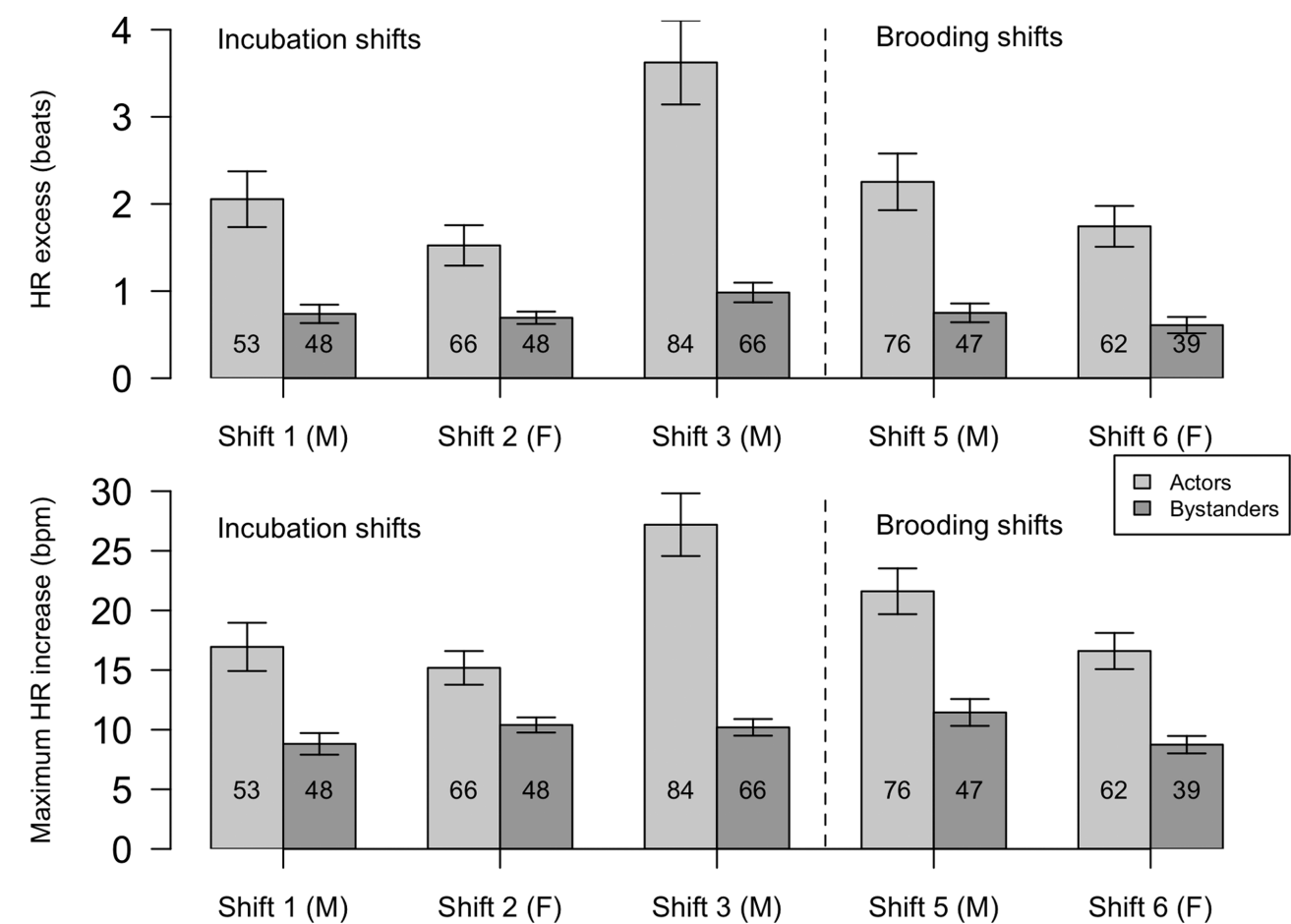
Figure 3
Variation in heart rate (HR) responses to agonistic interactions depending on the breeding status (incubation and brooding shifts) in male (M) and female (F) king penguins. HR excess and maximum increase as in Figure 1. Values are given as means ± SE for actors and bystanders (threats and blows were pooled together). Number of agonistic interactions is figured in the bars.

less vigorous physical activity. However, it is important to note that bystanders also exhibited substantial increases in HR as they witnessed interactions between neighboring birds, such increases not being attributable to physical activity because

they remained motionless. It may then be questioned to what extent HR changes are due to the stress experienced during agonistic interactions on the one hand, and physical exercise on the other (Viera et al. 2011). For instance, considering the

fact that maximal HR increases in bystanders were half those of actors, it may be that part of the observed HR increase in actors is due to experienced stress during agonistic interactions (Wascher et al. 2009). In addition, previous studies have reported the sympathetic nervous stimulation of the heart to show a slow response to physical activity (e.g., HR beginning to increase 1–2 s and reaching a plateau 30 s after the activity start in the domestic sow; Marchant et al. 1995). Thus, as in our study agonistic interactions were short-lived, and HR responses instantaneous and of a duration similar to that of the interaction, it is quite possible that HR increases may partly be caused by individual stress.

In addition, the risk associated with specific contexts may influence HR responses during aggressive interactions. Changes in physiological responses and HR according to individual evaluation of specific social contexts (including risk assessment) have indeed been documented in several species including geese (Wascher et al. 2009), pigs (Marchant et al. 1995), fish (Oliveira et al. 2005, 2001), and humans (social support; Steptoe 2000). In breeding king penguin, blows (high-risk interactions) provoked higher HR increases than threat displays (low-risk interactions), albeit the difference was only significant for actors. Thus, we cannot exclude the possibility that part of the difference observed might simply be due to higher physical activity for blows. For actors, HR responses also increased with the duration of the agonistic interaction and the rate of aggressive events during that interaction, both of which are associated with greater risk of injury and/or breeding failure. Again, we may not discard the possibility that physical activity might partly explain the observed pattern. However, it is interesting to note that in bystanders (motionless birds), the duration of interactions also significantly affected HR responses. In this case, HR increases could not be attributed to physical activity, and conflict duration might be a good proxy for assessing conflict risk for bystanders. Indeed, longer conflicts might imply greater risks of redirected aggression for bystanders, as redirected aggression is common in the king penguin and bystanding birds often end-up being involved in neighboring conflicts (personal observation). Overall, these results suggest that the evaluation of the context (whether the risk is high or low) may be important in modulating physiological responses to social stressors.

Our findings that HR increases in actors were significantly higher for initiators than for responders, even though the level of physical activity was not dissimilar between those 2 groups, suggest that individual motivation may be a strong modulator of HR responses during agonistic interactions. Interestingly, this effect was only significant for blows. Greater HR increases in initiators may then reflect cardiovascular changes brought about by individual preparation of engaging in physical blows (Adams et al. 1968), whereas low predictability and controllability of aggressions may constrain responses of targeted individuals (responders). Similar results have previously been reported for geese (Wascher et al. 2009). As in penguins, individual birds were shown to elicit higher HR increase when initiating rather than responding to agonistic interactions, suggesting that physiological (HR) responses to social stimuli in group-living animals may be affected by situation context or individual motivations (Wascher et al. 2009, see also Marchant et al. 1995; Sgoifo et al. 2001).

Finally, we found that HR responses of breeding penguins to agonistic interactions may vary depending on their breeding status. Indeed, we show that when actors of attacks, males relieving their partner as second-time incubators (males in shift 3) elicited significantly greater HR responses than did first-time incubators. Such a change in HR responses to social stressors may be linked to a change in the body condition of the animals, as costly stress responses may be downregulated in fasted animals (Kitaysky et al. 2005; Corbel et al. 2010). In our study, although all animals were monitored at the onset of an incubation/brooding shift, males in shift 1 would have typically been fasting for some 15 days more than males in shift 3, as the former would have been fasting during the courtship period before taking duty for the first incubation shift (Weimerskirch et al. 1992). Unfortunately, we did not measure body mass or structural size in our study, nor do we know the exact time at which birds started courting, so that future studies are needed to investigate the effect of the animal's condition on stress responses. Alternately, the finding that males' HR responses increased with advancing incubation may suggest a change in their sensitivity to the social environment and/or greater physical involvment in blows, possibly reflecting a change in parental commitment to the clutch as the relative reproductive value of the egg increases (Cézilly et al. 1994; Albrecht and Klvana 2004; Osiejuk and Kuczynski 2007). Indeed, according to parental investment theory (Trivers 1972), individuals may be willing to invest more into parental care as the clutch or offspring age, because of the higher probability of progeny of surviving until sexual maturity (Clutton-Brock 1991). If this were the case, one would have expected birds to display even higher HR responses during latter brooding shifts (when the egg has hatched), which does not appear to be the case. However, in our study, HR responses between chick-brooding birds and incubating birds may be confounded by the fact that they were monitored in different years, and our sample size per breeding shift is simply too low to draw any solid conclusions. It would be of interest for future studies to test this possibility for a larger sample of incubating and brooding birds during the same breeding season, including for females at shift 4 of incubation, the shift when hatching usually occurs (Stonehouse 1960), which we were unable to sample mostly due to time issues and to limitation in the number of birds we were allowed to handle.

To sum up, our findings provide evidence that HR responses to agonistic interactions in a colonial seabird depend both on the perceived relevance (and risk) of the interaction and on the motivation of the animal to engage into the interaction. They underline the importance of specific context assessment in modulating physiological responses to agonistic interactions in group-living species.

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