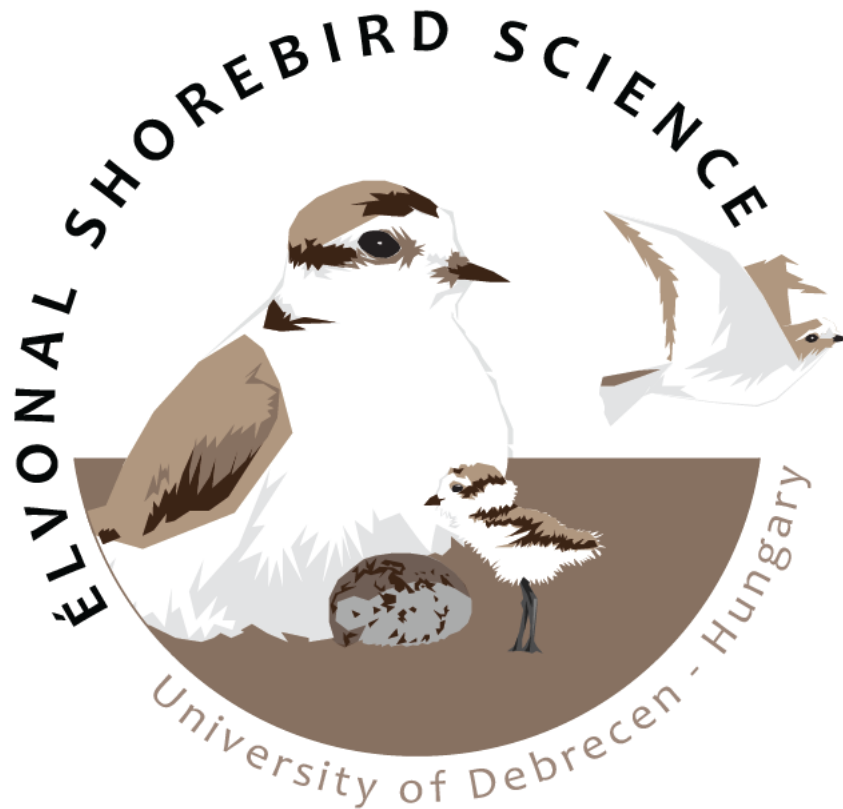


Protocol for collecting behavioural data for ÉLVONAL shorebird project

Tamás Székely & Vojtěch Kubelka, 12 February 2019

University of Debrecen (Hungary) & University of Bath (UK)



2019

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Contacts: T.Szekely@bath.ac.uk kubelkav@gmail.com

Background

The Hungarian government recently opened up a new funding stream “ÉLVONAL” to support cutting edge research across all scientific fields (Nature 551: 425), and our team was one of the 12 winners. Our project is focused on courtship, mating behaviour, pair bonding and parenting: social behaviours that are associated with breeding and often labelled collectively as sex roles (Schärer *et al.* 2012, Herridge *et al.* 2016). The main objective of this 5-year research project is to understand the evolutionary causes of sex role variation using shorebirds as model organisms, since shorebirds (sandpipers, plovers and allies) exhibit an unusual diversity of mating systems and parental care.

Our project has two main stages. In the first stage we are aiming to quantify sex role behaviour, and manipulate certain components in the field. In the second stage, we'll test demographic, genomic, immunological and disease-based causes of sex role variation. Our main hypothesis – based on previous works – is that adult sex ratio variation predicts sex roles, although the project will also explore alternative hypotheses. See references and a detailed description at <https://elvonashorebirds.com/>

This is a multi-population project: we hope to work with as many breeding shorebird populations as possible. Most of the data will be collected by core project participants, and although we also welcome external collaborators. The project will be coordinated by a Scientific Coordinator and a Research Assistant based at University of Debrecen, Hungary. We already established a network of communication system and a website <https://elvonashorebirds.com/> and in each year we plan an ÉLVONAL conference in Hungary that will focus on selected aspect of the project.

We hope our ÉLVONAL project will lead to high profile publications using breeding systems and sex role as core topics. We envisage 4–5 high profile papers and numerous smaller publications. Most of these will be coordinated by the ÉLVONAL team, although we are open to suggestion for collaborators to lead certain projects. We offer co-authorship to anyone who has made significant contribution to the projects. We hope that the ÉLVONAL project will stimulate further research into shorebird biology, have an impact on biodiversity conservation of shorebirds, train the next generation of shorebird biologists and conservationists, and foster new collaborations beyond the life-time of the ÉLVONAL project.

The objective of this document is to explain the behavioural data we seek to collect in the first stage of ÉLVONAL project (2018–2020).

Data collection

Collecting behavioural observation is lot more difficult than it seems. First, the observed bird must behave normally: that means you should use a hide (or car) to record visual observations or use a device (such as data logger or trail cameras) to record behaviour automatically. Do NOT attempt recording behaviour without appropriate cover. It takes usually 15–20 minutes for a shorebird to calm down once the observer enters his/her view. If you see that the observed shorebird displays antipredator display such as injury-feigning, has a stretched upright posture, runs or calls excitedly around the hide or a car, this means you are watching from too close. Do NOT record behaviour when the focal bird is apparently agitated due to your presence or to the research device you might be using.

Second, the observer should be far away not to disturb the focal bird(s) but close enough to see most behaviour and to identify individually the focal bird(s). The ideal observation distance varies between species, populations and habitats: being 50–200 m from the observed bird is the most common recording distance. If the observed bird disappears for a few minutes, do NOT stop recording its behaviour; rather, try to follow the focal bird and continue recording as precisely as you can, and mark the time on the observation sheet when the bird was out of your view (see below).

To collate behavioural observations from multiple populations, there are additional hurdles so that we need to think about standardizing data collection in advance. Achieving standardized data collection is immensely difficult, unless it can be automatized, since individual observers tend to have slight differences in observation and measurement methodologies. To gather meaningful and comparable data, we suggest:

1. each observer should read this BEHAVIOURAL PROTOCOL carefully. Please contact us if something is not clear;
2. each team should standardize data collection between their team members; this means observing the same focal bird simultaneously by different observers and then calculating repeatability across different observers;
3. during fieldwork and data collection, please ensure regular communication with the Scientific Coordinator, Research Assistant and other teams so that we share experiences and iron out hurdles as they are showing up.

We focus on four key aspects of sex roles (see below). Regardless of data collection methodology, for each behaviour data analyses will focus on percentage of time spent on sex-role related activities (the RESPONSE VARIABLES, see below). We request collaborators to provide these key data: % of time spent on sex-role related activities during an observational session.

We encourage each team to analyse, write-up and publish their own observations as specific research publications. This is entirely up to each team, and we could help if needed. In addition to these specific publications, we offer joint publications that will pull together data from multiple sites, species and behaviours.

RESPONSE VARIABLES

1. Courtship

Methodology

- use mated pairs that have not yet started incubation; i.e. looking for a nest site or in the process of completing their clutch;
- observe pairs in which at least ONE member of the pair is marked, but ideally, look for pairs in which BOTH the male and the female are individually marked;
- record 30 min observation for each pair using scan sampling on different days BEFORE or DURING egg-laying (see recording sheet below);
- target repeating the behavioural observations on 3 different days so that 3 × 30 min observations will be available for each focal bird;
- use robust behavioural categories, e.g. various display type, pre-copulatory displays (see below). For fights attempt to record whether the fight is with male, female or both.

The key variables that will be extracted from these records are % time spent on courting, displaying, copulating, and fighting with conspecific males & females; behavioural categories marked with ① in the descriptions below.

We recommend following the protocol used by Carmona-Isunza *et al.* (2015).

Additional variables to note for each behavioural record: species, location, date, time, male_ID, female_ID, behaviour of male and female at 20 sec for 30 min. Also, at every 5 min, record the estimated distance between male and female (see the attached field observation template at the end of this document).

2. Pair-bonding

Methodology

- identify BOTH individuals of pairs that produce a clutch or a brood of young;
- identification can be based on capturing both parents on the nest, although visual observations of the incubating (or brood-attending) parents are also acceptable;
- however, proximity to a nest or brood itself is not a fool-proof way of establishing pair-bonding; shorebirds, especially the colonial and semi-colonial nesters, move around nest and broods that they have no kin-relationship.

Key variables to extract: pair bonds between an individually identified male and a female in subsequent breeding attempts WITHIN a breeding season and BETWEEN breeding seasons.

Additional variables to record: species, location, nest, male_ID, female_ID, egg-laying date, nest success.

3. Parenting - incubation

Methodology

- identify individually marked pairs that are in the process of incubating their eggs;
- record 3 × 24 h observations using nest cameras or data loggers; these records can be on consecutive days accounting for 3 full days in total, although scattering the samples through whole incubation period is desirable to account for possible stage-dependent variation in incubation care and monitor some nests longer than 3 days.

Key variables to extract: % time the male, female or neither parent incubates the eggs; behavioural categories marked with ③ in the descriptions below.

We recommend following the protocol in Vincze *et al.* (2013).

Additional variable to record: species, location, nest, egg-completion date, date, time, clutch-size, male_ID, female_ID, behaviour of male and female at every 1 min.

4. Parenting – brood care

Methodology

- individually marked pair, or one adult only, that have a brood of chick(s);
- record 3 × 1 h observations during brood-rearing; these records should be on different days;
- use behavioural categories that relate to main parenting activities e.g. brooding, defending the young.

Key variables to extract: % time the male, female or neither parent brood or defend the young; behavioural categories marked with ④ in the descriptions below.

Variables to record: species, location, brood, hatch date, date, time, brood size, male_ID, female_ID, behaviour of parent(s) and chick(s) at every 30 sec. At every 5 min estimate the distance between the parent(s) and their chicks.

We recommend following the protocol in Székely & Cuthill (1999).

5. General background variables potentially to use in the analyses

We recommend using the Field guide for plovers (Székely *et al.* 2011) nest search, ringing, trapping and other aspects of fieldwork.

Key data needed for the ÉLVONAL project:

Parents: date of capture, nest ID, body mass, wing length, tarsus length, ring number, colour rings

Chicks: date of capture, nest ID, body mass, wing length, tarsus length, ring number, colour rings

Nests: completion date, hatch date, egg size and clutch size, nest exposure in days, nest fate

Blood samples from chicks and adults for paternity analyses

Ambient environment: ambient temperature, humidity, precipitation, wind speed, wind direction – every hour during the breeding season. These data need to be recorded during fieldwork, since recovering weather data from online resources is often problematic.

References

- Carmona-Isunza, M C et al. 2015. Courtship behavior differs between monogamous and polygamous plovers. *Behavioral Ecology & Sociobiology* 69: 2035–2042.
- Herridge EJ, Murray RL, Gwynne DT & Bussiere L. 2016. Diversity in mating and parental sex roles. In: Kliman RM (ed.). *Encyclopedia of Evolutionary Biology*, Oxford: Elsevier, pp. 453-458.
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Behavioural codes for shorebird observations

Tamás Székely & Vojtěch Kubelka, 12 February 2019

Recording behaviour in wild bird populations is not a trivial task, and although smart technology exists that replaces some of these observations (e.g. activity recorders, proximity sensors), to appreciate the rich natural history of shorebird behaviour it is best to be patient and watch behaviour directly using binoculars, a telescope and timer. It is not only fun but provide new ideas for follow-up studies.

Before starting a behavioural observation, please make sure that the birds behave normally. What you judge 'normal' may depend on how well you know the birds. Typically, after 15 – 20 min settling at a decent distance from the observed bird(s) will give you sensible behavioural records. If the observed birds (or a whole family) are alarmed and/or remain nervous, keep running away from you, circle around your hide or performing nest/brood defence towards you, these are clear signs that you are still disturbing them.

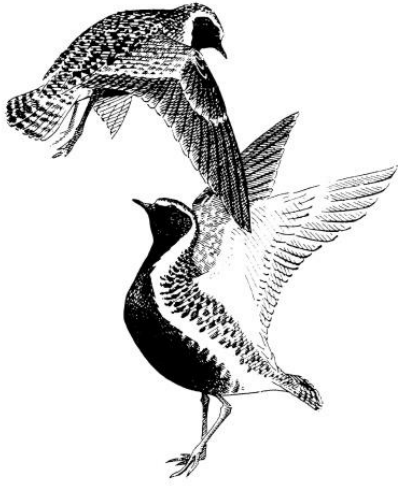
For observing one or two shorebirds (e.g., a courting couple), you should use scans at 20 sec intervals, whereas for three individuals or more (e.g., a family) it is better to take scans every 30 sec. It is also a good idea to note the distance between observed plovers (e.g. parents and chicks) at every 5 min.


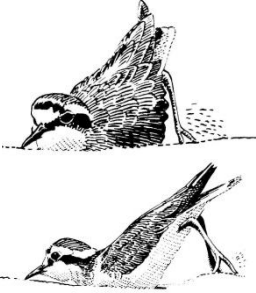
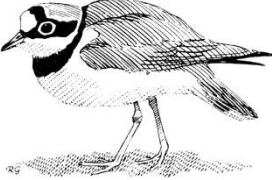

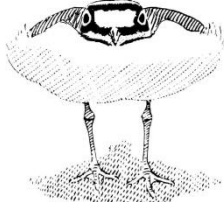
To take good scans you need to concentrate, and record the EXACT motion of the shorebird(s) at the time of the beep (one behaviour category per individual). If you watch a brood, it helps if you start the scans of all family members consistently in one direction, e.g. from left to right, and then swiftly write down the behavioural codes of each family member. DON'T THINK OR HESITATE – these may introduce error: just note what you saw. Remember that these behaviour categories are descriptive and we do not imply the function.


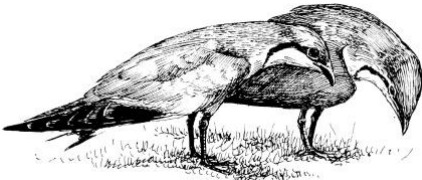

For courting pairs aim for 30 min observations on three different days, whereas for broods take 1 hour observation at a time. Repeat the observations every 3 – 4 day time interval until the chicks fledge or die.

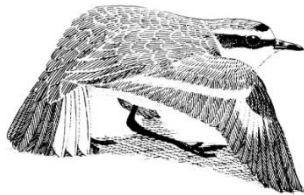

The list below provides the most common behaviour of shorebirds; hopefully these will cover most activities seen in shorebirds. If you see a behaviour that does not fit into any of these categories, feel free creating a new category and personalising these categories. Please define these new categories. Remember, by tweaking the behavioural categories below, you reduce the chance that your records will be comparable to those of collected by other observers. Remember, some of these behaviours can occur throughout the whole breeding season (e.g. maintenance), whereas others tend to be specific to certain reproductive stages (e.g. incubating the eggs).

Behaviour	Code	Comment
MAINTENANCE and FORAGING		
Walking	W	Walking individual, obviously slower movement than running.
Running	RU	Running individual, clearly on foot in movement.
Pecking/probing	PE	Pecking, typically, at a food item or probing (foraging technique typical for many sandpipers and allies).
Flying	FL	Bird is flying without any display.
Standing	STAND	Standing individual in relaxed position, often on one leg or with hidden head.

Looking above	LOOK	Looking by one eye above, often in relaxed position – scanning sky for potential danger.
Standing head-up	HU	Alerted individual, often scanning the surrounding for potential danger.
Standing extreme head-up	EHU	Alerted individual with stretched neck (vertically elongated), often scanning the surrounding for danger.
Sitting	SIT	Sitting individual (sometimes with hidden head) on the ground, not nest. This is different from incubation.
Preening	PR	Maintenance of feathers (often ruffled) by bill.
Head scratching	HS	Head scratching by leg as a part of maintenance behaviour.
Wing stretching	WS	Wing stretching as a part of maintenance behaviour.
Leg stretching	LS	Leg stretching as a part of maintenance behaviour.
Head flicking	HF	Head flicking to the side (over the shoulder).
Drinking	DRINK	Often repeated dipping of the bill to the water, drinking.
Bathing	BATH	Bathing in the shallow water with ruffled feathers, feathers soaking and often wings flapping.
Body shaking	BS	A movement lasting a few seconds of body shaking with ruffled feathers, often after bathing or stretching.
Tail shaking	TS	Tail shaking, often after bathing and body shaking.
Ring pecking	RP	Pecking aimed at the metal or colour rings, trying to remove them, often observed in freshly ringed individuals.
Fighting conspecific (unknown sex) ① Fighting conspecific male ① Fighting conspecific female ① Fighting heterospecific individual (note species and the sex if possible)	FIC FICM FICF FIH	 <p>Longer aggressive physical contact between two individuals. Note whether with a male or a female, AND with conspecific or other species. This behaviour is not a part of an attack during nest or brood defence (see later categories).</p>
Chasing conspecific (unknown sex) ① Chasing conspecific male ① Chasing conspecific female ① Chasing heterospecific individual (note species and the sex if possible)	CHC CHCM CHCF CHCH	A quick aggressive behaviour against another animal, typically a waterbird species as well to chase it away from the feeding ground – not as a part of an attack during nest or brood defence. Despite the fight where two individuals fight against each other, here one individual is chasing the other which is trying to escape.

COURTSHIP		
Aerial display ①	AER	 <p>Display in flight, typically by male. Often slow flight, wings fluttering, often accompanied with characteristic voice. Mainly plovers and sandpipers.</p>
Scraping ①	SCRAPE	 <p>Movement lasting several seconds, typically performed by male (but the female can also be involved), when an individual is making a scrape by pushing its' breast to the ground and scraping its' legs. Males are regularly preparing several scrapes in the territory in this way, predominantly when female is watching nearby.</p>
Hunched-back running ①	HRU	 <p>Running typically nearby possible mate in crouched position with hunched back.</p>
Hunched-back standing ①	HST	Standing in the same position as described above.
Pecking nest material ①	PENE	Pecking nest material.
Pre-copulation ①	P-COP	Male balances on female's back prior copulation.
Copulation ①	COP	 <p>Act of mating with cloacal contact of two adult individuals, when male is on female's back, often accompanied with characteristic vocalization.</p>
Flat running ①	FRU	 <p>Running with the head and back at the same level, often feathers fluffed to the sides.</p>
Flat standing ①	FST	Standing with the head and back at the same level, often feathers fluffed to the sides.
Throwing nest material to the nest ①	THNE	Picking up nest material and throwing it towards the nest scrape (typically backward over body) during the courtship or to the nest with clutch, often after incubation recess.

Wing display ①	WIDI	 <p>Wing showing, tail showing to the potential mate on the ground, typical behaviour for several sandpiper species.</p>
Choking courtship ①	CHOK	 <p>Close intimate courtship of two individuals (current or possible future pair), often accompanied by typical vocalization (e.g. “choking”).</p>
Ground display ①	GRO	Displaying behaviour of the male at the ground or from suitable post in the breeding territory, often accompanied with characteristic vocalization. Also lekking display of Ruff or Great Snipe.
INCUBATION		
Incubation ③	INC	Directly sitting on the nest to incubate eggs.
Shading of eggs ③	SHADE	Standing above the nest, shading eggs, leaving wind cooling eggs down a little bit during hot part of the day.
Eggs wetting ③	WET	Visitation of nearby water body, belly-soaking and eggs wetting to cool them down.
Eggs covering ③	COV	Covering eggs with sand or vegetation pieces prior departure from the nest.
BROOD ATTENDANCE		
Brooding the chick(s) ④	B	 <p>Chick(s) is/are at least partially hidden in adult’s breast feathers and under wings. Adult can stand or sit on the ground. Typically during cold or extremely hot environmental conditions.</p>
Shading the chick(s) ④	SH	Chick(s) is/are just sheltered (NOT brooded) by standing adult, typically with stretched wings (usually during hot environment conditions.
NEST OR BROOD DEFENCE – against natural predators or local humans, but not against observing researchers		Make always a note towards which potential predator the particular behaviour is performed.
Distant calling ④	DC	Calling/warning from distance, typically from the ground, giving potential predator away, but without closer approach to the predator.

Injury feigning ④	IF	 <p>Adult is pretending to be injured, e.g. “broken wing display” “wing flapping”, resembling easy prey for potential predator, to allure potential predator away from real nest location. Also impeded flight or erratic fluttering belongs here.</p>
Rodent running ④	RRU	Rapid running in crouched position with the head tilted downwards, the body plumage fluffed, and the wings slightly extended and dropped, often accompanied by squeaking. Bird pretends to be a small rodent (a suitable prey) alluring potential predator away from real nest location. Regular behaviour of many Arctic sandpipers.
False brooding ④	FB	Adult is pretending to sit on the nest at different location to allure potential predator away from real nest location.
Other displacement activities ④	DIAC	False feeding, false swimming, pseudo-sleeping or false maintenance behaviour as a part of the distraction display to allure potential predator away from real nest location.
Crouched running ④	CRU	Contrary to rodent run – silent run in crouched position rather reducing conspicuousness of bird departing from nest, behaviour typical for many plovers.
Upright running ④	URU	Distractive running which does not involve crouched posture, typically with head-up.
Ungulate display ④	UNG	 <p>To deter a big animal (often ungulate) from vicinity of nest or brood, adult shorebird faces the animal with fully raised wing(s) and loud calling.</p>
Aggressive circling and scolding ④	AC	Intense flight above or in close vicinity of approaching predator whilst emitting alarm calls (mobbing). Without physical contact.
Attack ④	AT	Intense mobbing connected with physical contact with intruder/predator, often repeated and predominantly air strikes complemented with loud vocalization. Also special “snake pecking” belongs here. Always note which predator is involved and whether other individuals from the surrounding helped the focal defender.
OTHER		
Covered	C	Focal bird is covered from view, e.g. behind vegetation.
Away	A	Focal bird is away, e.g. not around the brood.

Numbers in the table denote particular behavioural categories – sex roles specific activities performed during courtship ①, incubation ③ and brood care ④. Drawings were adapted from Cramp S. and Simmons K.E.L. (eds). 1983: *Handbook of the Birds of Europe, the Middle East, and North Africa: The Birds of the Western Palearctic, Volume III: Waders to Gulls*, Oxford Univ. Press.

Since behaviour depends so much on weather, it is essential to have weather data near the timing of behavioural observation. If you can't get weather data, it would be desirable to at least note ambient temperature, precipitation and wind.

We recommend using the following sheets for Courtship and Brood care. For species that have 4 chicks, an extra column need to be added to the latter form.

Sp: Year: Date: Start: End:
 Site: UTM: M_ID: F_ID: Observer:

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Comments:

Sp: Year: Date: Start: End:
 Site: Fam: Parents: Chicks: Observer:

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