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Research Article

Is a visuo-haptic differentiation of zebra mussel and quagga mussel based on a single external morphometric shell character possible?

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Abstract

The sharp-angled (presence of an acute ridge) or rounded (absence of an acute ridge) transition of the ventral and dorsal shell surfaces is the "key feature" for the distinction of *Dreissena polymorpha* and *Dreissena rostriformis*. However, up to now it has not been possible to put this into a quantifiable framework. Therefore, the aim of this study was to develop a method, which (i) facilitates the quantification of this feature as a morphometric parameter and (ii) to test whether the presence or absence of an acute ridge can be used to distinguish the two species both in mathematical terms and under field conditions. We called the new parameter 'angularity' (*An*) and developed a method for its quantification. $An \leq 3.3$ and $An \geq 4.7$ were the discriminant thresholds for quagga mussels and zebra mussels, respectively. $3.3 < An < 4.7$ did not allow for a clear distinction between species. Nevertheless, *An* is highly sensitive in morphometrically discriminating both species: more than 96% of the mussels were correctly classified whereas less than 1% were falsely classified. We conducted a visuo-haptic experiment in which we asked test persons to rate mussels according to their angularity (acute ridge present *vs*. absent). Remarkably, our results revealed that all participants were well able to distinguish the species at an error rate of 8.4 %. Nevertheless, even naïve persons who have no previous knowledge of dreissenid mussels can reliably select specimens of zebra and quagga mussels under field conditions based on the angularity alone.

Key words: *Dreissena polymorpha, Dreissena rostriformis*, biometrics, DNA barcoding, signal-detection approach

Introduction

The zebra mussel (*Dreissena polymorpha,* Pallas, 1771) and the quagga mussel (*Dreissena rostriformis*, Deshayes, 1838, also *D. rostriformis bugensis,* Andrusov, 1897, taxonomic revision by Stepien et al. 2014), are native to the Ponto-Caspian Region (Son 2007). They are important invasive species in freshwater systems of the Northern Hemisphere. Both species live in similar habitats and have similar life-history characteristics. In their native range, they often co-occur, but they differ in timing and rates of spread, habitat requirements, growth rates and population dynamics (Karatayev et al. 2014). The expansion of the zebra mussel started in the late 18th century to the north-west (Kinzelbach 1992). Currently, its invasive area stretches over large parts of Europe (van der Velde et al. 2010) and North America (Bossenbroek et al. 2007; Brown and Stepien 2010), where it has successfully established large populations. In contrast, the range expansion of the quagga mussel was much slower. Its initial eastward expansion did not start until the 1940s when ecosystems were changed or created by river modification and the formation of dams, and when natural geographic barriers were removed by construction of irrigation canals (Orlova et al. 2014). In the late 1980s, it was introduced into North America (Benson 2014) and within the last ten years it has appeared in Western Europe (bij de Vaate et al. 2014; Orlova 2014; Paulus et al. 2014).

The recent and asynchronous range expansions of these two ecologically similar species places them in direct competition for available resources (Zhulidov et al. 2010). Outside its native range, *D. rostriformis* appears to be competitively superior to the zebra

mussel. In cases where it invades areas, where *D. polymorpha* is already established, it often competitively displaces the latter within a few years as the dominant dreissenid species (Orlova et al. 2005; Ram et al. 2012; Wilson et al. 2006). Both species have attracted great attention not only because of their outstanding ability to colonise new habitats and water bodies, but also due to their great impact on aquatic communities (Nalepa et al. 2009; Ward and Ricciardi 2014).

When *D. polymorpha* (*Dp*) and *D. rostriformis* (*Dr*) first appeared together in North America, May and Marsden (1992) described diagnostic external shell features of both species, which were further elaborated by Pathy and Mackie (1993). Additional characteristics were subsequently described by e.g. Claxton et al. (1998), Heiler et al. (2011), Marescaux et al.(2012), and Pavlova and Izyumov (2014). The most commonly used diagnostic characters are the sharp-angled (*Dp*) versus rounded (*Dr*) transition of the ventral and dorsal shell surfaces and the concave/flat (*Dp*) versus convex (*Dr*) ventral shell surface (e.g. Molloy et al. 2007; Peyer et al. 2011; Zhulidov et al. 2010). However, the pronounced plasticity in shell morphology of both species severely hampers species discrimination (Ram et al. 2012). In the quagga mussel, this problem is further increased by the occurrence of the deep-water phenotype "profunda" (Claxton et al. 1998; Pavlova 2012; Peyer et al. 2010). Consequently and according to Stepien et al. (2014), most morphologists make some errors in distinguishing dreissenid species.

An unambiguous distinction for *D. polymorpha* and *D. rostriformis* is crucial for the reconstruction of their invasion routes, which in turn is needed to successfully facilitate mitigation strategies for controlling or preventing invasions (Estoup and Guillemaud 2010). Furthermore, *D. polymorpha* is widely used in biomonitoring programmes (e.g. Alcaraz et al. 2011; Paulus et al. 2015; Riva et al. 2010) whereas *D. rostriformis* is still being tested as a monitoring organism (Johns 2011; Mueting and Gerstenberger 2010) and hitherto has only rarely been used for biomonitoring (Richman and Somers 2010). Since both species significantly differ in their potential to accumulate environmental contaminants (Richman and Somers 2005; Rutzke et al. 2000; Schäfer et al. 2012), a reliable differentiation is essential.

In contrast to morphology, zebra and quagga mussels can unambiguously be identified using genetic markers (Feldheim et al. 2011; Hoy et al. 2010; Marescaux and Van Doninck 2013; Stepien et al. 1999). However, in biomonitoring programs it is necessary to discriminate the two species in large quantities and under field conditions. Therefore, reliable external diagnostic shell features are urgently needed. Ram et al. (2012) designated the sharp-angled or rounded transition of the ventral and dorsal surfaces as the "key feature" for distinction of the two species. However, under field conditions its application is highly subjective and observer dependent. A recent attempt, albeit based on low sample sizes, to test if well trained researchers are able to discriminate between genetically determined specimens of *D. polymorpha* and *D. rostriformis* found error rates for pattern and shape characteristics (6% and 25% respectively), which are too high to be reliably applied during field identification (Beggel et al. 2015).

Therefore, the aim of our study is to develop an approach that allows quantification of the morphometric discriminant power of the shape of the transition between the dorsal and ventral shell side. Given its importance for species determination under field conditions, we further test to which degree even *Dreissena*-naïve observers can use this character for both visual and haptic discrimination in a way that it can be used as the sole external morphometric shell feature to distinguish zebra and quagga mussels in the field.

Methods

Sampling

The German Environmental Specimen Bank (ESB) samples *D. polymorpha* annually at 14 sampling sites located at German rivers (Elbe, Rhine, Saar and Danube) and from Lake Belau based on standard operating procedures (Wagner et al. 2003). We provided genetic evidence for the occurrence of *D. rostriformis* in all river systems (Paulus et al. 2014 and unpublished data for the river Saar; see Figure 1).

For our model data set, we sampled *D. polymorpha* and *D. rostriformis* in October 2012 from the river bank beneath the low water line at D2. We selected this locality because both species exhibit a highly variable shell morphology at this site. For the purpose of gaining validation data one combined set of zebra and quagga mussels was each sampled from three sites located in different river systems where it was known that the species co-existed (D3, E4, R3; see Figure 1). Samples were gathered from the river banks at D3 and E4 and from exposed plate stacks of the ESB at R3.

Genetic analysis

DNA was extracted from a small piece of soft tissue using the Qiagen DNEasy Blood & Tissue Kit and following the manufacturers' protocol. A ca. 460 bp

portion of the mitochondrial 16S-rDNA gene was sequenced for all specimens using the versatile primers 16Sar-L and 16Sr-H of (Palumbi et al. 2002). After initial melting at 94°C for 120 s, 39 cycles at 94°C for 30 s, 41°C for 90 s and 65°C for 60 s were run for PCR amplification, with a final cycle at 72°C for 180 s. PCR products were purified using the Roche High Pure PCR Product Purification Kit following the manufacturers protocol. Cycle sequencing with 16Sar-L was done by MacroGen company. Sequences were edited and aligned using Mega (version 6, (Tamura et al. 2013). Reference sequences were downloaded from GenBank: AF038996 (*D. rostriformis*), AF038997 (*D. polymorpha*), AF038998 (*Mytilopsis leucophaeata*) and AF038999 (*Corbicula fluminea*) (Stepien et al. 1999). Species assignment of test specimens was done by blast search via Mega as well as by constructing a simple neighbor joining tree using the uncorrected *p*-distance and 2000 bootstrap replicates. By each of the two approaches, all specimens could be unambiguously assigned to either of the two species.

Morphometric approach

We defined the size-independent quantitative parameter "angularity" (*An*). The morphometric quantification of the angularity of *D. polymorpha* and *D. rostriformis* was based on the anterior-posterior shell view (Figure 2 and 3). *An* was determined separately for the left and the right valve. Firstly, the valves were divided by a vertical line where the valves meet. Next, the point with the maximum perpendicular distance between this dividing line and the shell contour was denoted "*CC*" (centre of the circle). If there was no single point, but rather an equidistant section of the contour, *CC* was specified as the point which was furthest away and at the edge where the contour started to narrow towards the ventral side. The distance from the central line to the point *CC* was denoted "*W*" (width). For *D. polymorpha*, the contour curve at point *CC* generally has a distinct kink, whereas the contour of *D. rostriformis* in the direct vicinity of *CC* ideally shows no kink and more closely resembles the arc of a circle with its centre located on the central line and with radius *R*. Basically, the idea behind this method was to measure the angle of this kink and relate it to what was expected for such a circle. To do this in a way that is independent of the mussel's size, a circle with the radius *W*/5 was centred in *CC*. The intersections (*I*) of the circle with the contour were defined as *I*1 and *I*2. Thus, *I*1 and *I*2 lie a distance *W*/5 away from *CC*. Connecting lines from *CC* to *I*1 and *I*2 were drawn and the angle, *A*, was measured between the two lines. If the contour of the mussel was like a circle centred on the central line, the angle would also be non-zero. In fact, it would be approximately 11.48° determined by the formula: $A = 2sin^{-1}(1/10)\ 360/2\pi \approx 11.48$. Thus, the normalised relative angle, *An*, was determined as the measured angle, *A*, divided by the angle obtained for the circle: *An* = A/11.48. Determined *An*-values of *D. rostriformis* were expected to be lower (closer to one) than those obtained from *D. polymorpha*, which should show significantly higher values.

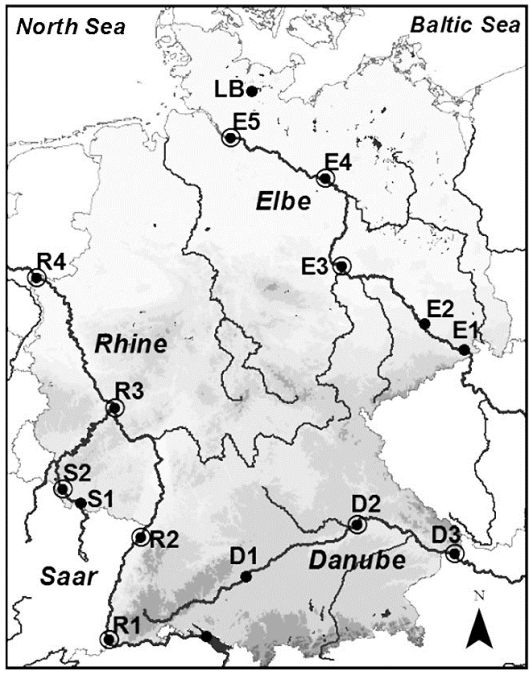

Figure 1. *Dreissena polymorpha* sampling sites of the German ESB (E= Elbe, R = Rhine, S = Saar, D = Danube, LB = Lake Belau). Framed dots are sampling sites with genetic evidence of co-occurring *D. rostriformis*.

Figure 2. *Dreissena polymorpha* (left) and *Dreissena rostriformis* (right) in the anterior-posterior view. Arrows indicate the part of the valves under examination (transition between dorsal and ventral shell surfaces).

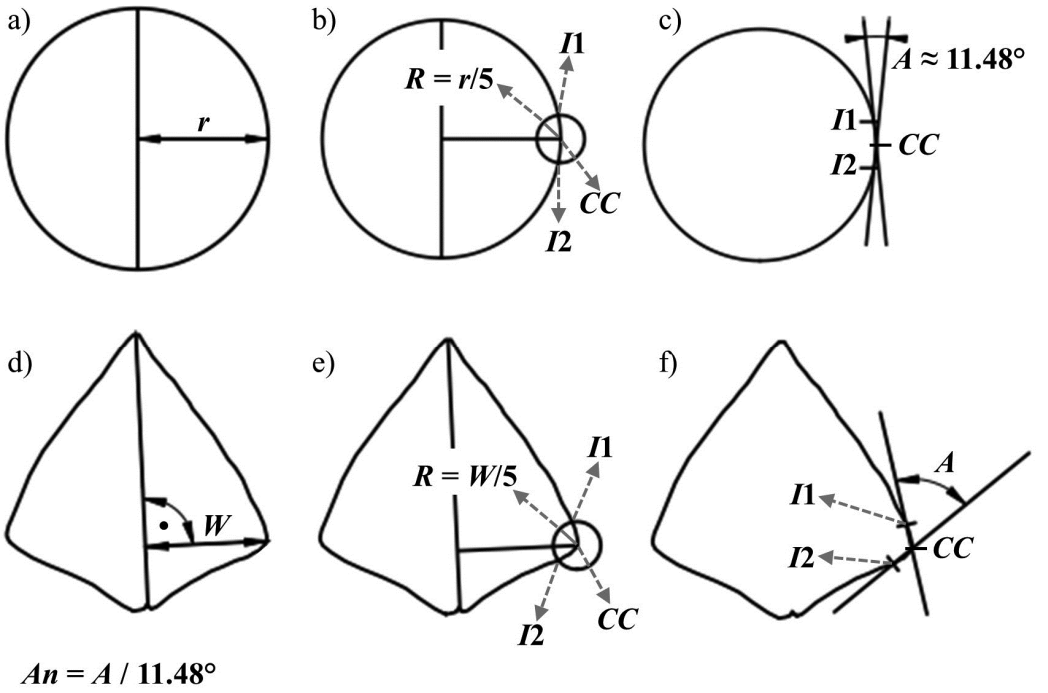

Figure 3. Quantification of the angularity (*An*) of *Dreissena* mussels with r = *R* = radius, *I* = intersection, *A* = angle, *W* = width, *CC* = centre of the circle: a) *r* is the radius of the initial circle. b) *R* is the radius of the circle with the centre *CC*, *I*1 and *I*2 are the intersections of the circle of *R* = *r*/5 with the initial circle. c) Connecting lines between *I*1 and *CC* plus *I*2 and *CC*, *A* is the angle between the two lines ≈ 11.48°. d) Vertical line between the valves, *W* is the maximum perpendicular distance between vertical line and shell contour. e) *CC* is the point on the shell contour with *W*, *W*/5 is the radius of the circle with centre *CC*, *I*1 and *I*2 are the intersections of the circle of *R* = *W*/5 with the contour. f) Connecting lines between *I*1 and *CC* plus *I*2 and *CC*, *A* is the angle between the two lines.

Valves were cleaned, dried and agglutinated. The shells were locked into an upright position in the anterior-posterior view of the mussels by placing them onto a bed of putty and then aligned to an angle iron. Photographs were taken with a single lens reflex camera. These pictures were used to measure the angle *A* (see Figure 3). All measurements were carried out with RibbonSoft QCad 1.5.1.

A canonical linear discriminant analysis was conducted to assess how well angularity acts as a distinguishing criterion for the species and, furthermore, to derive discriminant values. The analysis was conducted with IBM SPSS 22.

Visual and haptic perception of the angularity

We tested whether naïve persons could perceive the presence or absence of an acute ridge at the transition of the ventral and dorsal surfaces. To this end, 32 *Dreissena*-naïve students from Trier University individually participated in an experiment. They were presented with one mussel at a time, and their task was to visually and haptically explore the mussel. The same task-instructions were read to each participant by an experimenter. The participants were instructed to consider both valves of a mussel to make the decision. Further, it was emphasised that the experiment aimed to examine the validity of the shaping of the mussels rather than the individual's ability to discriminate the mussels. Participants were asked to respond as fast and accurately as possible. To familiarise with the task, the participants performed 20 practice trials with mussels clearly identifiable as *D. polymorpha* (acute ridge with $An \geq 5.05$) or *D. rostriformis* (rounded with $An \leq 2.96$). All participants were presented with the same mussels with the order of presentation randomised across participants. To this end, all mussels were numbered and the order of number presentation was controlled using the programming software E-Prime (version 2.0). That is, the experimenter, who was seated in front of a computer screen, was presented with the number of the mussel to be handed to the participants at the beginning of each trial. Importantly, the experimenter did not know the correct answer (acute ridge present *vs*. absent) at that time. The participant then explored

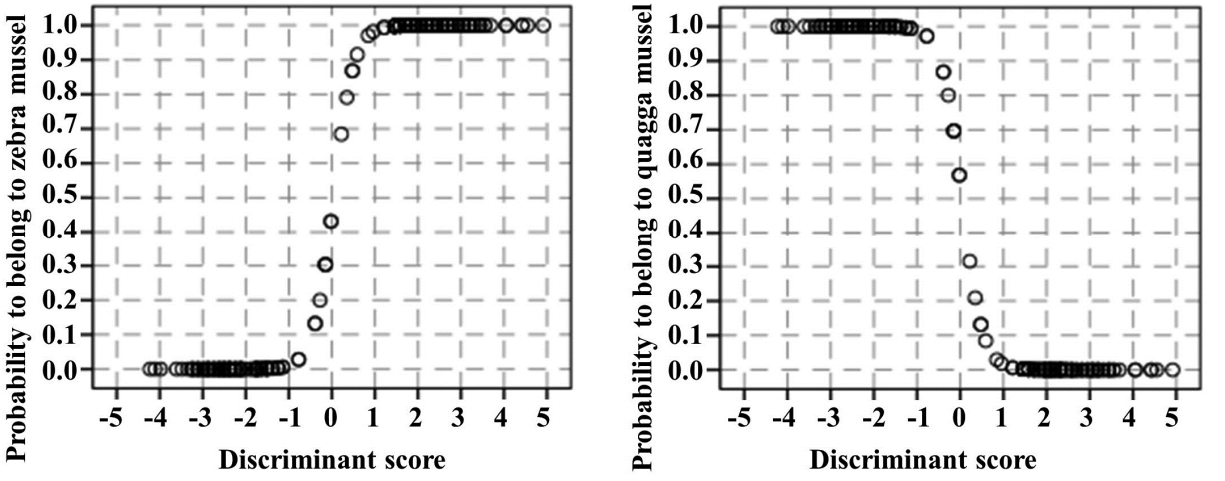

Figure 4. Plot of the discriminant scores of the discriminant function resulting from the *An*-values against the probabilities of group membership.

the mussel and verbally responded "Yes" if it was perceived to be sharp-angled or "No" if it was perceived to be rounded. The experimenter coded the participant's answer by pressing either the J-key (if the participant had answered "Yes") or else the N-key on the keyboard. During the practice trials, the experimenter was then presented with feedback on the computer screen ("This was the right answer" or "Unfortunately, this was the wrong answer"), which he or she read aloud to the participant. Then the next trial started. After the practice trials, participants had a short break before the start of 209 experimental trials. The only difference between the practice and the experimental trials was that participants received no feedback during the experimental phase.

Within the experimental phase, participants were presented with 104 *D. polymorpha* and 105 *D. rostriformis* mussels. Again, the order of presentation was random and controlled by E-Prime. Importantly, the experimenter took the mussels out of a kind of shelf. Here, the mussels were not ordered according to their species. Still, to prevent influences of the position of a mussel in the experimental set-up on the participant's responses, the experimenter and the participants sat on different sides of an opaque shield, and the experimenter handed the indicated mussel over the shield for the participant to explore it, and took it back after the participant responded before starting the next trial. Note that there were 1-min pauses after every 26 trials. One participant had to be excluded from the analysis because she did not follow the task instructions (i.e., by self-report, did not focus on the mussels' angularity, leading to an error rate of 47.1%).

The obtained data were analysed using a signal-detection approach (SDT; (Swets 1964)). The advantage of this approach is that it can separate response bias (i.e., participants' tendency to say "yes", for example) and discrimination performance. Note that the participants in the current experiment made binary decisions (is there an acute ridge or not?) under uncertainty (i.e., at the beginning of a trial, participants had no information about the angularity of the mussel at hand). Importantly, a mussel could either belong to the species of *D. polymorpha* (where there is an acute ridge, i.e., signal present) or to the species of *D. rostriformis* (where there is no acute ridge, i.e., signal absent). Since the participants were forced to make binary decisions, a given mussel was to be judged either as possessing an acute ridge or not.

Results

Morphometric approach

The discriminant analysis for the model data set could correctly classify 97.1% of the originally grouped mussels (97.1% of the zebra mussels and 97.2% of the quagga mussels) (Eigenvalue = 4.783; canonical correlation = 0.909; Chi-square = 360.626; significance $p < 0.001$).

For the determination of the discriminant values, the discriminant scores of the discriminant function resulting from the *An*-values were plotted against the probabilities of the *An*-values belonging to either the zebra or quagga mussel group (Figure 4).

If the discriminant score was +1 or higher, then the probability that the *An*-value indicated a zebra

Table 1. Classification results of the valves based on the derived discriminant values (Z = zebra mussel, Q = quagga mussel).

Sampling site	Species	n	Correctly classified		Non-classified (Intermediate)		Falsely classified	
			count	%	count	%	count	%
D2	Z	102	96	94.1	5	4.9	1	1.0
D2	Q	106	97	91.5	9	8.5	0	0.0
D3	Z	44	44	100.0	0	0.0	0	0.0
D3	Q	42	41	97.6	1	2.4	0	0.0
E4	Z	40	35	87.5	5	12.5	0	0.0
E4	Q	42	41	97.6	1	2.4	0	0.0
R3	Z	42	34	80.9	6	14.3	2	4.8
R3	Q	40	39	97.5	1	2.5	0	0.0

Table 2. Classification results of the mussels based on the derived discriminant values (Z = zebras mussel, Q = quagga mussel).

Sampling site	Species	n	Correctly classified		Non-classified (Intermediate)		Falsely classified	
			count	%	count	%	count	%
D2	Z	51	50	98.0	0	0.0	1	2.0
D2	Q	53	50	94.3	3	5.7	0	0.0
D3	Z	22	22	100.0	0	0.0	0	0.0
D3	Q	21	21	100.0	0	0.0	0	0.0
E4	Z	20	19	95.0	1	5.0	0	0.0
E4	Q	21	21	100.0	0	0.0	0	0.0
R3	Z	21	18	85.7	1	4.8	2	9.5
R3	Q	20	20	100.0	0	0.0	0	0.0

mussel was approximately 99% (Figure 3, left). On the other hand, if the discriminant score was -1 or lower, it was a quagga mussel with a probability of approximately 99% (Figure 3, right).

An was subsequently regressed against the discriminant scores to obtain the regression equation: $An = 4.025 + 0.704 \times$ discriminant score. Based on this equation *An* for the discriminant scores +1 and -1 was calculated: $An\ (+1) = 4.7$ and $An\ (-1) = 3.3$. Thus, $An \leq 3.3$ and $An \geq 4.7$ were the discriminant values for quagga mussels and zebra mussels, respectively. The intermediate interval between these two values did not allow for a clear designation to either species. Values of the calculated angularity were then assigned to one of the three groups and subsequently checked for correct classification (Table 1).

92.8% of the values of the model data set were correctly classified, 0.5% falsely and 6.7% could not be assigned. 93.6% of the values of the summarised validation data sets were correctly classified, 0.8% falsely and 5.6% could not be classified.

The classification of these values was performed notwithstanding of the fact that a mussel consists of two valves. However, there is a need to classify the whole mussel and not only single valves. Since there are three possibilities for the discriminant value of each valve i.e. belonging to the quagga, zebra or intermediate section, we have six possibilities for the entire mussel. There are the following possibilities for the combination of two valves: both valves are classified as zebra mussel (ZZ) or quagga mussel (QQ); both values gives opposite results (ZQ); both valves belong to the intermediate section (II); one valve is classified as zebra or quagga mussel and the other one belongs to the intermediate section (ZI or QI). If the discriminant value of the two valves gives opposite results, the mussel cannot be classified. Likewise, the same applies for the combination of two intermediate values. If one valve has an intermediate value, the other valve is used for the classification (Table 2).

The discriminant value of the two valves never gave opposite results in either the model data set or

the validation data set. We note that in the entire data set, if the two valves were both non-intermediary, the mussel was never falsely classified. 96.1% of the mussels from the model data set were correctly and 1% was falsely classified; 2.9% of the mussels could not be assigned properly. 96.8% of the mussels of the summarised validation data sets were correctly and 1.6% falsely classified; 1.6% could not be classified at all.

Visual and haptic perception of the angularity

In our experiment, participants were instructed to categorise a given mussel as either having an acute ridge (say "yes") or not (say "no"). Given that we presented the participants with quagga mussels and zebra mussels in a random order, there hence were four possible outcomes for each classification. Specifically, there were hits (the participant says "yes" in response to a zebra mussel), misses (the participant says "no" in response to a zebra mussel), false alarms (the participant responds "yes" in response to a quagga mussel), and correct rejections (the participant responds "no" in response to a quagga mussel). Note that the genetic constitution of each mussel was used for species identification. In this classification, hits and correct rejections reflect good performance, whereas false alarms and misses reflect failure.

Generally, the performance level was very high. On average, 91.6 % (standard deviation, *SD* = 6.0 %) of mussels were correctly classified (hits and correct rejections) either as *D. polymorpha* and *D. rostriformis*. The response patterns were analysed in more detail, revealing that classification performance was above chance for each participant at an individual level (for each participant significance < 0.001; Chi-square values ranging from 45.541 to 197.220, with a mean of 145.076, and a *SD* of 42.234). Further, we compared the average error rate (ER) considering those mussels with two distinct valves (ZZ, QQ, n = 184, ER = 6.73%, SD = 5.54 %), on the one hand, and those mussels that were hard to classify (ZI, QI, n = 20, ER = 16.94 %, *SD* = 12.63%), on the other, by means of an independent-samples test using Students *t* as a criterion. Note that the data met the precondition of this test that the difference score ($ER_{ZZQQ} - ER_{ZIQI}$) is normally distributed (p = 0.878). Remarkably, ER was significantly lower for those mussels with two, as compared to only one, distinct valves, $t_{(30)} = 5.727$, $p < 0.001$. Additionally, the average performance level was lower for those mussels that could not be classified based on the angularity of both valves (II, n = 5, ER = 35.48 %, *SD* = 19.12 %) as compared to ZI and QI mussels, $t_{(30)} = 6.096$, $p < 0.001$. We also compared the error rates of zebra (n = 104, ER = 8.78%, *SD* = 11.93%) and quagga mussels (n = 105, ER = 8.02%, *SD* = 8.97%). There were no significant differences between both species ($t < 1$).

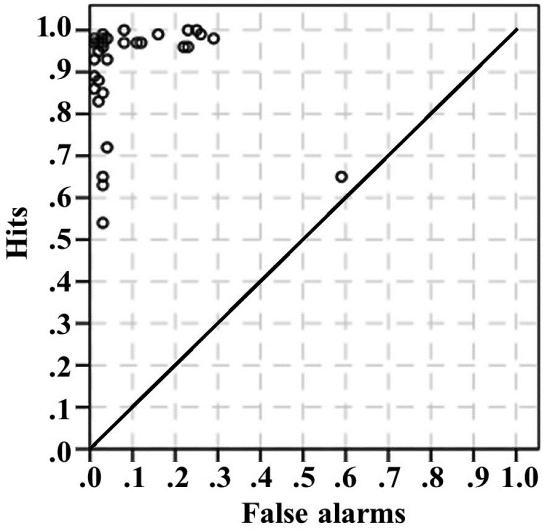

Figure 5. Plot of the individual hit rates against the individual false alarm rates. The solid line indicates performance at chance level. Perfect classification is indicated by a hit rate = 1 and a false alarm rate = 0.

To more clearly analyse the data, we computed the signal detection parameter *d'*. Note that we followed the so-called log-linear approach (see Hautus 1995; Stanislaw and Todorov, 1999), which involves adding 0.5 to both the number of hits and the number of false alarms and adding 1 to both the number of signal trials and the number of noise trials, before calculating the hit and false alarm rates. The average *d'* was significant (with individual *d'* ranging from 1.917 to 4.028, with a mean of 3.060, *SD* = 0.574; overall $t_{(30)} = 29.697$, $p < 0.001$). That is, participants were sensitive to judging the mussels' angularity. As an illustration, the participants' performance is depicted in Figure 5.

Note that the present signal-detection approach is a simplification of the participant's exploration, since there was variance in the mussel's angularity. In contrast, the stimulus intensity is typically held constant (and on a low level) in signal-detection experiments. Hence, we also analysed *d'* separately for ZZ-QQ mussels, ZI-QI mussels, and II mussels. The analyses revealed that the classification of ZI, QI and II mussels was still above chance (i.e., $p < 0.05$) for 24 out of the 31 participants included in the analysis (Chi-square values overall ranging from 1.52 to 18.360, with a mean of 9.252, *SD* = 4.517).

Further, *d'* was still significant when only these mussels were considered (mean $d'_{\mathrm{ZI\ QI\ II}} = 1.640$, $SD = 0.624$; $t_{(30)} = 14.640$, $p < 0.001$. In more detail, *d'* was significant for those mussels hard to identify (mean $d'_{\mathrm{ZI\ QI}} = 2.644$, $SD = 0.759$; $t_{(30)} = 19.399$, $p < 0.001$) and also for those mussels not identifiably based on the angularity model ($d'_{\mathrm{II}} = 0.637$, $SD = 0.805$, $t_{(30)} = 4.404$, $p < 0.001$). Yet, $d'_{\mathrm{ZI\ QI\ II}}$ was significantly lower than *d'* for ZZ and QQ mussels (mean $d'_{\mathrm{ZZ\ QQ}} = 3.429$, $SD = 0.684$; $t_{(30)} = 27.902$, $p < 0.001$), $F(1,30) = 250.800$, $p < 0.001$, partial eta² = 0.893).

Discussion

We successfully developed a method for the morphometric quantification of the presence or absence of the kink in the shell contour of *D. polymorpha* and *D. rostriformis*, turning a rather subjective morphological criterion into an objectively quantifiable character. The results of our discriminant analysis show that the angularity is highly sensitive in discriminating *D. polymorpha* and *D. rostriformis*. They exceed the results of Beggel et al. (2015) who, even with a combination of three morphometric measurements, could only achieve a canonical correlation of 0.855 and a degree of correct classification of 94.4%. Claxton et al. (1997) also were able to differentiate between zebra and quagga mussels using a single shell characteristic. However, they used the degree of overlap between the left and right valve, a character that is limited to juveniles, with shell length of approximately 300–700 µm.

Importantly, as illustrated by Figure 4, the angularity does not determine a single discriminant value, which distinguishes the two species from each other. Rather it defines the lower and upper thresholds (discriminant values for either species) of a transitional interval between the two species, where assigning the values to either species is not possible ("intermediate interval"). The classification results of the model data set (Table 1) support our approach with two discriminant values: Only a small fraction of less than 1% of the values were falsely classified, whereas almost 93% were correct. The presence of both zebra and quagga mussels in the intermediate group further corroborates the definition of an intermediate angularity interval. These results were confirmed by those of the validation data sets.

When considering a mussel as a whole, consisting of two valves, also less than 1% of the mussels were falsely classified. In contrast to the results of the single valves, more than 96% of the complete mussels were correctly classified. This was validated by the results of the three other sampling sites, although here the falsely classified proportion increased and the intermediate group decreased by less than 1%. These minor changes were caused by the fact that all falsely classified valves showed intermediate *An*-values. Thus, not only the single valve but also the entire mussel was assigned to the wrong species. However, considering the mussels as a whole slightly increased model power because a larger fraction of the intermediate group was then classified. An erroneous species classification occurred in only three cases across all data sets (a zebra mussel was classified as quagga mussel). In contrast, quagga mussels never showed angularities in the range of zebra mussel. Therefore, it is sound to assume that the only apparent type of error when applying angularity is the nonidentification of a kink and thus the exclusively misclassification of zebra mussels as quagga mussels, although at a small rate (2.6%). Other authors reported higher misclassification rates for quagga mussels based on three different morphometric parameters (Beggel et al. 2015). This may be due the higher variability of shell proportions of quagga mussels compared to zebra mussels (Pavlova and Izyumov 2014). However, this seems not to affect the classification rate of quagga mussels based on angularity. In applications where it is not admissible to have falsely classified mussels it is therefore necessary to exclude mussels with one intermediate valve as not classified. The overall loss of mussels would be less than 4%.

In addition to the morphometric quantification, we conducted an experiment in which we asked participants to dichotomously rate mussels according to their angularity (acute ridge present *vs*. absent). Remarkably, our results revealed that all participants focussing on the mussels' angularity as the discriminant feature were very well able to distinguish the species. Thus, even persons who had no previous knowledge on dreissenid mussels can discriminate zebra and quagga mussels based on both visual and haptic perception of the angularity. These findings are contrary to those of Beggel et al. (2015) who found the transition angle between the dorsal and ventral side highly subjective. However, they used only seven test persons and 36 mussels; hence, the power of their results is lower than that of our study with 32 test persons and 209 mussels.

With regard to the visual and haptic exploration, the test persons performed significantly better for mussels with both valves exceeding/falling below the defined morphometric thresholds. Forcing participants to judge the mussels' angularity resulted in an overall error rate of 8.4%. This slightly limits the applicability of the visuo-haptic approach for field identification. In cases where the relative abundances of the two species has to be determined in the field,

the visuo-haptic approach is still valid, since a similarly small proportion of both species would be falsely classified due to comparable error rates.

In conclusion, if a researcher relies on the visually and haptically perceived angularity of a mussel in the field, she or he will be able to reliably select specimens of *D. polymorpha* and *D. rostriformis*. However, it still remains open if and to which degree the classification rate of field researchers can be improved by training their visual and haptic perception of the mussels' angularity.

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