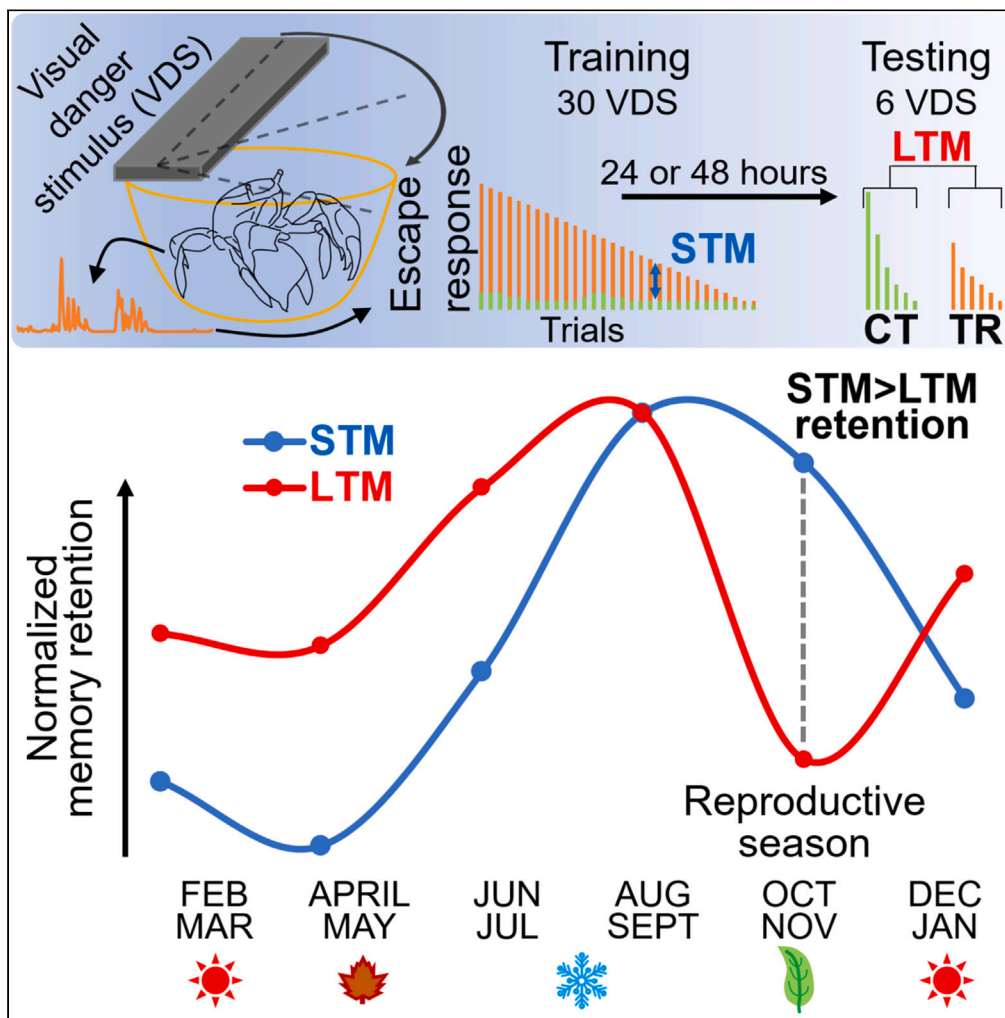


Article

Annual changes of *Neohelice granulata* cognitive abilities indicate opposition between short- and long-term memory retention



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Highlights

Short- and long-term
memory retention
aptitudes change along the
year

Memory retention abilities
indicate a trade-off
between short- and long-
term memory

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Article

Annual changes of *Neohelice granulata* cognitive abilities indicate opposition between short- and long-term memory retentionRosso Anahi¹ and Freudenthal Ramiro^{1,2,*}

SUMMARY

Neohelice is a long-standing model for memory studies for its strong retention of a reduced escape response when trained to iterative presentations of a visual danger stimulus (VDS). Here we present year-round changes that are related to the memory acquisition, storage, and expression. First, we evaluated exploratory activity and response to the VDS, as necessary for memory acquisition and expression. Both parameters change year-round. Second, short-term memory (STM) and two types of long-term memory (LTM) were assessed throughout the year. STM and long-term context-dependent signal memory (CSM) change between periods of the year, whereas signal memory (SM) does not, indicating that the cognitive abilities of the crab display circannual rhythms. Third, during the reproductive period, STM retention is higher than both CSM and SM, indicating a trade-off between STM and LTM. This is the first report of memory retention abilities changing seasonally as a trade-off between short- and long-term memories.

INTRODUCTION

The cognitive abilities of the crab *Neohelice granulata* are object of intense interdisciplinary studies that focus on appetitive and avoidance learning, memory consolidation and reconsolidation, neural information computing, molecular bases of memory, and the biological relevance of behavioral strategies.^{1–4} *Neohelice* crabs are predated by seagulls in the coastline of the San Clemente del Tuyú estuary.⁵ In line with this fact, the visual stimulation produced by an object moving above triggers an escape response in the crabs. Under iterative presentations of a visual danger stimulus (VDS), a persistent reduction of this defensive response is observed, shifting from a conspicuous escape response to a general reduction in movement. Previous results have shown that for this change in the escape response crabs use information acquired during the repeated exposure to the VDS that is stored as memory. The long-term reduction of the escape response is a context-specific habituation;⁶ evidence of this associative feature in habituation models has been reported in mammals including humans, birds, and invertebrates.⁷ An extensive study was performed to ascertain the possible biological meaning of the robust memory capacities of the crab.⁶ In the laboratory, changes in the escape response and the retention of this change in behavior over time are evidenced as differences between a control and trained crabs (control [CT] and trained [TR] groups respectively). The reduction of the escape response during the training session is an expression of short-term memory (STM).⁸ A TR group median escape response significantly lower than the median activity of CT group when comparing the sum of the last 10 trials of the training session (Tr21–30) indicates a strategy shift that requires the use of short-term memory.⁹ The long-term memory (LTM) expression of this strategy shift lasts for at least 5 days,¹⁰ and it can be divided into two main components: the context-dependent signal memory (CSM) and the signal memory (SM). The CSM is the main responsible for a significantly higher response being observed in the CT animals (exposed to the context during training but without VDS) than in the TR animals (exposed to context-VDS presentations during training) during the VDS presentation of the first evaluation trial (Ts1). For the second type of retention, the SM is mostly responsible for the TR group having significantly lower escape response than the CT group when comparing medians of the summed responses of testing trial 2 to 6 (Ts2–6).^{6,9} When studying CSM requirements, an observation is that animals during the spring months October and November show low long-term memory retention, a period that coincides with the start of the mating and reproductive period of *Neohelice granulata*, reported from October to February.¹¹ This suggested, as reported for other animal species, a circannual rhythm affecting the cognitive capabilities of *Neohelice granulata*.^{12–14} Also, it has been hypothesized that cognitive abilities are important in mating. Recently a link has been reported between spatial memory retention, a critical element for mating success, and the ability to acquire and defend a territory, indicating a key role for cognition in mating.¹⁵ Using *Neohelice granulata*, Santos et al., 2021,¹⁶ highlight the social context as a modulator of cognitive abilities; the fight outcome between two male crabs modulates associative memories, and long-term memory retention of subordinates is higher than that of their respective dominant. This suggests a possible link between long-term memory

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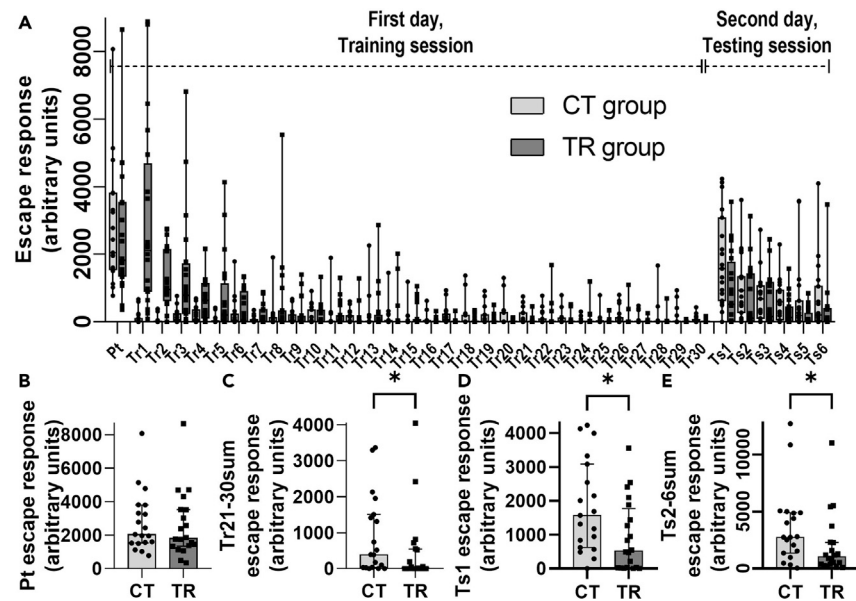


Figure 1. Crab escape responses during training and testing sessions. Individualization of the parameters quantified

(A) Training and Testing sessions of a single pair of CT and TR animal groups. Box and whiskers, IQR, min-max, showing all animals as points. CT (n = 19) TR (n = 20).

(B) Escape response during pre-training (Pt) to the first VDS exposure (FRV).

(C) Sum of trials 21 to 30 of the escape responses registered during training (Tr21-30).

(D) Escape response of the first trial of the testing session.

(E) Sum of trials 2 to 6 of the escape responses of the Testing session. As the data were not normal, Mann-Whitney test was used to evaluate the significance of differences. $p < 0.05$ *.

retention level and the capacity to tackle agonistic encounters between *Neohelice granulata* males, which is one of the strategies that affect mating success during the reproductive season.¹⁷ Here we report the variations of two sensorimotor and three memory retention parameters along the year: the exploratory activity (Ea) and the first escape response to the VDS (FRV), as sensorimotor parameters of the crabs, and short-term memory expressed during training and the long-term CSM and SM as mnemonic parameters. This was achieved using as a unit the CT and TR groups of crabs that were trained and evaluated in the experimental device at the same time, for each of the periods studied, for several years. The results show circannual variations for all the parameters except the long-term SM. The results suggest a trade-off between short- and long-term memory that coincides with the onset of the reproductive season. These findings indicate that the observed changes between short and long-term memory can be influenced by circannual rhythms and may be triggered by the cognitive requirements of a demanding mating environment.

RESULTS

Neohelice granulata escape strategy shift evidence acquired information use

The motor activity of individual animals from CT and TR groups during the experimental protocol could be organized to evidence sensorimotor parameters that represent the crab's general state and mnemonic parameters that through evaluation of memory retention indicate how the animals use the new information in short- and long-term memories (Figure 1).

The Figure 1A displays the animal's responses during the exposure to the behavioral apparatus during training and testing sessions. After the first exposure of all animals to VDS, a waning escape response in the TR group is evident when compared during the same trial with the CT group. Comparison with the CT group (without VDS during training trials) throughout the iterative exposure to VDS shows a clear learning curve in the TR group. During the 6-trial evaluation session (VDS exposure for both groups) animals of the TR group show significantly lower escape responses than CT animals. As the data were not normal, Mann-Whitney tests were used to evaluate significant differences.

The escape responses of CT and TR groups during the first exposure to the VDS are not significantly different, $U = 168$, $p = 0.55$ (Figure 1B), indicating that response differences between groups were not present before training. The results indicated that Tr21-30 sum of TR animals is significantly lower than Tr21-30 sum of CT animals, $U = 119$, $p = 0.0427$ (Figure 1C), indicating a change in the escape strategy of TR animals and short-term memory retention. During the first trial of the testing session, TR group escape response is significantly lower than CT group escape responses, $U = 111$, $p = 0.0257$ (Figure 1D), showing long-term CSM retention. Testing trials 2 to 6 show that Ts2-6 sum of TR animals is significantly lower than TR2-6 sum of CT animals, $U = 115$, $p = 0.0353$ (Figure 1E), signifying long-term SM retention. These results show short- and long-term memory retentions of a usual pool of animals that were trained at the same time.

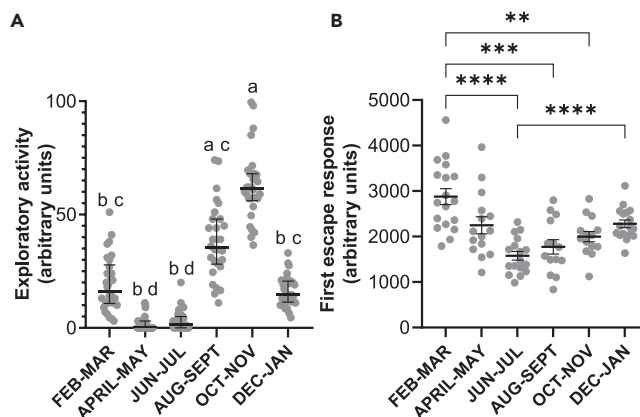


Figure 2. Annual changes in sensorimotor parameters

(A) Exploratory activity. Motor activity of CT group animals registered 30 times for 50 min. Each point is the median of all animals registered in the period. Median, IQR. For all variables with the same letter, the difference between the medians is not statistically significant. $p < 0.05$.

(B) First escape response. Response measures for all animals during pre-training presentation of VDS. Mean and SEM. $p < 0.01$ **, $p < 0.001$ ***, and $p < 0.0001$ ****.

To understand the annual changes observed in memory retention traits, we analyzed first the sensorimotor responses of the crab that represent previous traits of the animals that affect the measurement or expression of memory retention and could be affected by seasonal variations of the environment. In this line of thought we explore possible correlations to environmental parameters as well as species-specific variations year-round, to then evaluate the annual changes in retention.

Sensorimotor responses

Ea

The Ea of the animals was assessed for every run of TR session using the responses of the animals of the CT group during the 50 min duration and 30 recordings that co-occurred at the same time as the VDS was presented to the animals of the TR group (CT group of Figure 1A during training session and plotted for every period in Figure 2A). The Ea is as follows: FEB-MAR median 16 IQR 27.75–10.75, $n = 247$; APRIL-MAY median 0.000 IQR 3.000–0.000, $n = 167$; JUN-JUL median 1.5 IQR 5–0, $n = 175$; AUG-SEPT median 35.25 IQR 48–28.13, $n = 146$; OCT-NOV median 61.50 IQR 68.00–56.13, $n = 184$; DEC-JAN median 14.5 IQR 20.63–11.38, $n = 246$ (Figure 2A). The Ea differences of the 30 recordings for every two months during the year were evaluated for significance with a non-parametric repeated measures Friedman test, Friedman statistic 133.4, p value < 0.0001 , number of groups 6. A Dunn's multiple comparisons test indicates the following significant differences among medians: FEB-MAR vs. APRIL-MAY, $p < 0.0001$; FEB-MAR vs. JUN-JUL, $p = 0.0019$; FEB-MAR vs. OCT-NOV, $p < 0.0001$; APRIL-MAY vs. AUG-SEPT, $p < 0.0001$; APRIL-MAY vs. OCT-NOV, $p < 0.0001$; APRIL-MAY vs. DEC-JAN, $p < 0.0001$; JUN-JUL vs. AUG-SEPT, $p < 0.0001$; JUN-JUL vs. OCT-NOV, $p < 0.0001$; JUN-JUL vs. DEC-JAN, $p = 0.0025$; OCT-NOV vs. DEC-JAN, $p < 0.0001$.

The observed variation in Ea could be grossly resumed in a period of low Ea during the 4-month period between April and July, a higher Ea in the following 4 months between October and November, and a period of intermediate Ea in the 4 months between December and March (Figure 2A).

FRV

The other sensorimotor parameter that could affect the evaluation of memory retention responses around the year is the intensity of the response to the VDS. To evaluate the annual variation of this parameter, we used the response to the first presentation to the VDS during the pre-training presentation used to distribute the animals in the CT and TR groups (Figures 1A and 1B). The initial escape response shows a clear annual variation (Figure 2B), mean, SEM, and number of runs for each period: FEB-MAR, 2,875, 175.0, 18; APRIL-MAY, 2,248, 187.2, 15; JUN-JUL, 1,576, 95.07, 17; AUG-SEPT, 1,773, 158.3, 14; OCT-NOV, 1,999, 113.4, 14; DEC-JAN, 2,280, 85.37, 17.

A Brown-Forsythe ANOVA test, F^* (DFn, DFd) 11.34 (5.000, 89.31), p value < 0.0001 , shows a significant difference among means. Tamhane's T2 multiple comparisons test indicates the following significant differences among means: FEB-MAR vs. JUN-JUL, $t = 6.521$, degrees of freedom (DF) = 26.10, $p < 0.0001$; FEB-MAR vs. AUG-SEPT, $t = 4.671$, DF = 29.97, $p = 0.0009$; FEB-MAR vs. OCT-NOV, $t = 4.202$, DF = 27.85, $p = 0.0037$; JUN-JUL vs. DEC-JAN, $t = 5.506$, DF = 31.64, $p < 0.0001$.

The FRV shows a maximum in FEB-MAR and a minimum in the JUN-JUL period.

Data indicate that both Ea and FRV change annually and that these variations should be considered to assess the annual memory retention shifts.

Memory retention parameters

As the sensorimotor responses vary annually, we use indexes (see STAR Methods) that relativize the responses to the FRV to compare memory retention aptitudes between periods of the year and direct comparisons between CT and TR animal responses to evaluate the presence of retention.

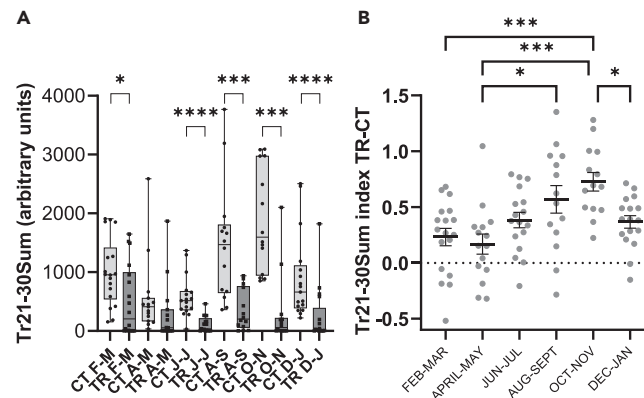


Figure 3. Annual changes in Short-term strategy shift

(A) Sum of Tr21-30, CT vs. TR animals by period. Escape strategy shift during training session. Box and whiskers. Median, IQR, Max-min.

(B) Annual variations in strategy shift during acquisition. Pt-Tr21-30 index difference (TR-CT). Mean and SEM. Asterisk indicates significant differences between CT and TR for each period in A and between periods in B. $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***; $p < 0.0001$ ****.

Short-term strategy shift evaluation Tr21-30 sum

The first mnemonic parameter to be addressed is the difference observed between CT and TR animals in the sum of the last 10 trials of the training session, in which the trained animals evidence a shift in the escape strategy displayed during the VDS presentation (Figures 1A and 1C).

As this shift occurs during the learning curve and after 30 min of 20 VDS presentations, this difference indicates an expression of short-term memory.

To evaluate the changes in Tr21-30 sum, in the pairs of CT-TR for each experimental date in the 6 annual periods evaluated (Figure 3), we first used independent Wilcoxon matched-pairs signed rank tests (Wilcoxon) as shown in Figure 3A. For FEB-MAR, Wilcoxon indicated that CT F-M was statistically significantly higher than TR F-M $W = 30$, $N = 18$, $p = 0.0139$. For APRIL-MAY, Wilcoxon indicated that CT A-M was not statistically significantly different from TR A-M $W = 36$, $N = 15$, $p = 0.1876$. For JUN-JUL, Wilcoxon indicated that CT J-J was statistically significantly higher than TR J-J $W = 3$, $N = 18$, $p < 0.0001$. For AUG-SEPT, Wilcoxon indicated that CT A-S was statistically significantly higher than TR A-S $W = 4$, $N = 14$, $p = 0.0009$. For OCT-NOV, Wilcoxon indicated that CT O-N was statistically significantly higher than TR O-N $W = 0$, $N = 14$, $p = 0.0009$. For DEC-JAN, Wilcoxon indicated that CT O-N was statistically significantly higher than TR O-N $W = 2$, $N = 17$, $p < 0.0001$.

A one way ANOVA was used to evaluate differences in acquiring this strategy shift (TR-CT differences in the TR21-30 index) between periods of the year, $f = 5.922$, $p < 0.0001$ (Figure 3B). Holm-Sidak's multiple comparisons test indicates significant differences in these comparisons: FEB-MAR vs. OCT-NOV $p = 0.0009$; APRIL-MAY vs. AUG-SEPT $p = 0.0202$; APRIL-MAY vs. OCT-NOV $p = 0.0003$, and OCT-NOV vs. DEC-JAN $p = 0.0385$.

It is important to keep in mind that the change in the escape response between the first presentation of the VDS (median Pt, TR group 1,815, Figure 1B) and the summed response of trials 21–30 (median Tr21-30 sum 7.5 TR group, Figure 1C) is always evident. What is observed with this variable is that the summed escape response of the last 10 training trials for the TR group is significantly less than the sum (of the same 10 trials) of the Ea of the CT group (median of Tr21-30 sum 397, CT group, Figures 1, 3A, and 3B). This difference between CT and TR animals is observed in all periods except APRIL-MAY (Figure 3A), and the level of the shift in strategy observed between CT and TR animals is significantly different between the periods of the year studied, showing the highest levels in the OCT-NOV period (Figure 3B).

Long-term strategy shift CSM Ts1

The first trial of the testing session shows the long-term memory retention of the CSM (Figures 1A and 1D). To evaluate the changes in Ts1, in the pairs of CT-TR for each experimental date in the 6 annual periods evaluated, we first used paired t tests when the data were normal and independent Wilcoxon matched-pairs signed rank tests when not normal, as shown in Figure 4A.

For FEB-MAR, paired t test indicated that CT F-M was statistically significantly higher than TR F-M $t = 4.195$, degrees of freedom (df) = 17, $p = 0.0006$. For APRIL-MAY, Wilcoxon indicated that CT A-M was statistically significantly higher than TR A-M $W = 10$, $N = 15$, $p = 0.0026$. For JUN-JUL, paired t test indicated that CT J-J was statistically significantly higher than TR J-J $t = 5.124$, df = 16, $p = 0.0001$. For AUG-SEPT, paired t test indicated that CT A-S was statistically significantly higher than TR A-S $t = 7.316$, df = 13, $p < 0.0001$. For OCT-NOV, Wilcoxon indicated that CT O-N was statistically not significantly different than TR O-N $W = 23$, $N = 14$, $p = 0.0676$. For DEC-JAN, paired t test indicated that CT O-N was statistically significantly higher than TR O-N $t = 4.883$, df = 16, $p = 0.0002$.

The escape response TS1 index median, IQR, and number of runs for each period (Figure 3B) are as follows: FEB-MAR, 0.2253, 0.3695–0.03468, 18; APRIL-MAY, 0.3256, 0.4559–0.1150, 15; JUN-JUL, 0.3866, 0.6581–0.1947, 17; AUG-SEPT, 0.4225, 0.8105–0.3254, 14; OCT-NOV, 0.1245, 0.2839–(–0.07210), 14; DEC-JAN, 0.4789, 0.7499–0.2476, 17.

A Kruskal-Wallis test indicated significant differences in acquiring this strategy shift (TR-CT differences in the Ts1 index) between periods of the year, $K-W = 21.21$, $p = 0.0007$ (Figure 4B). Dunn's multiple comparisons test indicates significant differences in these comparisons: JUN-JUL vs. OCT-NOV $p = 0.0332$; AUG-SEPT vs. OCT-NOV $p = 0.0053$; OCT-NOV vs. DEC-JAN $p = 0.0141$.

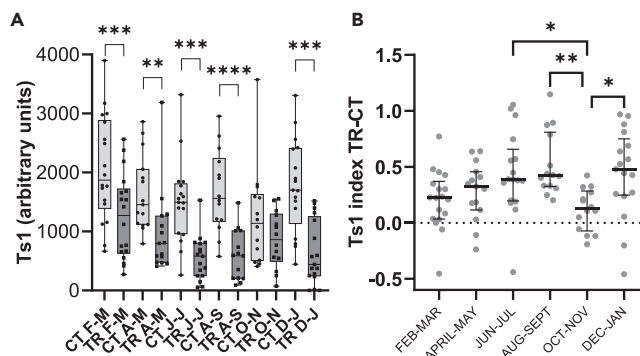


Figure 4. Annual changes in Long-term context-dependent signal memory retention

(A) First trial of testing session, CT vs. TR animals by period. Escape strategy during the first trial of the testing session. Box and whiskers. Median, IQR, Max-min. (B) Annual variations in context-dependent signal memory retention. Pt-Ts1 index difference (TR-CT). Median and IQR. Asterisk indicates significant differences between CT and TR for each period in A and between periods in B. $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***; $p < 0.0001$ ****.

A strong context signal long-term memory retention measured as a significant difference in response to the VDS on the Ts1 between CT and TR animals is clear in all periods except in the OCT-NOV, where there are no significant differences (Figure 4A). There is a variation in the probability of detecting this memory retention along the year. The OCT-NOV Ts1 retention is significantly lower than JUN-JUL, AUG-SEPT, and DEC-JAN. The OCT-NOV period overlaps with the onset of the reproductive season, suggesting that is a factor affecting this memory retention.

Long-term strategy shift SM Ts2-6 sum

The sum of the trials 2 to 6 of the testing session shows the long-term memory retention of the SM (Figures 1A and 1E).

To evaluate the changes in Ts2-6 sum, in the pairs of CT-TR for each experimental date in the 6 annual periods evaluated, we first used paired t tests when the data were normal and independent Wilcoxon matched-pairs signed rank tests when not normal, as shown in Figure 5A.

The escape response for the Ts2-6 sum of CT and TR groups, mean, and SEM for normal periods and median and IQR for non-normal periods (Figure 5A) are as follows: FEB-MAR, CT: 4,444, 436.9, TR: 2,049, 217; APRIL-MAY, CT: 2,540, 304.1, TR: 1,509, 256.2; JUN-JUL, CT: 2,014, 242.8, TR: 640.5, 97.16; AUG-SEPT, CT: 2,605, 3,811–1,425, TR: 782.5, 1,358–515; OCT-NOV, CT: 2,726, 3,337–2,072, TR: 1,696, 2,499–950.8; DEC-JAN, CT: 2,932, 293, TR: 1,623, 245.7.

For FEB-MAR, paired t test indicated that CT F-M was statistically significantly higher than TR F-M $t = 5.528$, $df = 17$, $p < 0.0001$. For APRIL-MAY, paired t test indicated that CT A-M was statistically significantly higher than TR A-M $t = 4.415$, $df = 14$, $p = 0.0006$. For JUN-JUL, paired t test indicated that CT J-J was statistically significantly higher than TR J-J $t = 4.731$, $df = 16$, $p = 0.0002$. For AUG-SEPT, Wilcoxon indicated that CT A-S was statistically significantly higher than TR A-S $W = 0$, $N = 14$, $p = 0.0001$. For OCT-NOV, Wilcoxon indicated that CT O-N was statistically significantly higher than TR O-N $W = 0$, $N = 14$, $p = 0.0001$. For DEC-JAN, paired t test indicated that CT O-N was statistically significantly higher than TR O-N $t = 3.725$, $df = 16$, $p = 0.0018$.

The escape response Ts2-6 index median and IQR for each period (Figure 5B) are as follows: FEB-MAR, 0.3391, 0.5266–0.1091; APRIL-MAY, 0.2397, 0.4436–0.09458; JUN-JUL, 0.4349, 0.8454–0.1640; AUG-SEPT, 0.5557, 0.8387–0.2954; OCT-NOV, 0.1738, 0.3646–0.1293; DEC-JAN,

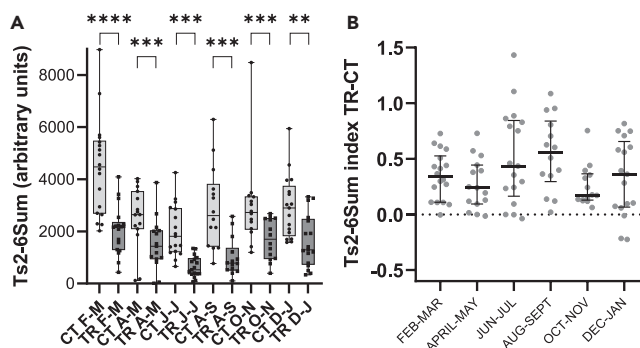


Figure 5. Annual long-term signal memory retention

(A) Sum of trials 2–6 of testing session, CT vs. TR animals by period. Escape strategy during the trials 2–6 of the testing session. Box and whiskers. Median, IQR, Max-min. (B) Annual variations in signal memory retention. Pt-Ts2-6 index difference (TR-CT). Median and IQR. Asterisk indicates significant differences between CT and TR for each period in A and between periods in B. $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***; $p < 0.0001$ ****.

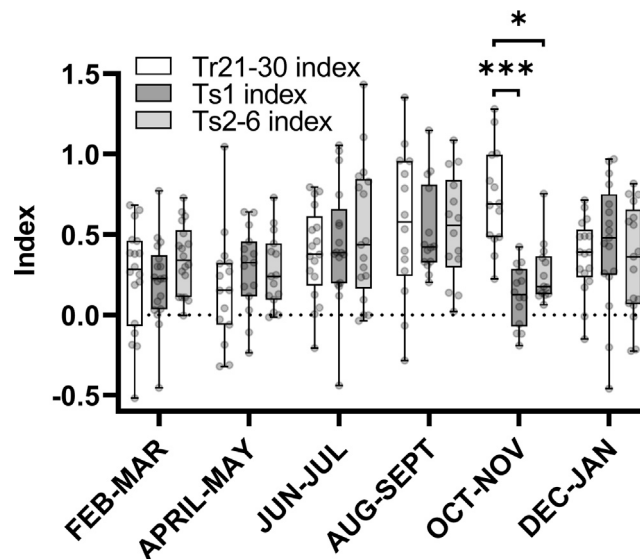


Figure 6. Memory retention profile of each period during the year

Memory retentions of STM (Pt-Tr21-30 index difference TR-CT), CSM (Pt-Ts1 index difference TR-CT) and SM (Pt-Ts2-6 index difference TR-CT) for each period of the year. Box and whiskers. Median, IQR, Max-min. Asterisk indicates significant differences between memory retention types within each period. $p < 0.05$ *; $p < 0.001$ ***.

0.3610, 0.6559–0.06635. A Kruskal-Wallis test indicates that there are no significant differences between the medians of the SM retention index (Ts2-6 index) between the periods of the year evaluated, $K-W = 8.338$, $p = 0.1386$ (Figure 5B).

Within periods, retention of STM (Tr21-30), CSM (Ts1), and SM (Ts2-6) memories, expressed as a CT group response significantly higher than the response of TR group, is observed in all periods, with two exceptions (Figures 3A, 4A, and 5A). During APRIL-MAY Tr21-30 and during OCT-NOV Ts1, both show no retention (Figures 3A and 4A).

Between periods, significant differences are reported for Tr21-30 index and for Ts1 index, but not for Ts2-6 index (Figures 3B, 4B, and 5B). These results indicate circannual variations in sensorimotor responses Ea and FRV as well as in the short-term memory retention evaluated in Tr21-30 and the long-term CSM evaluated in the Ts1.

Learning indexes comparison for each period of the year

To compare memory retention between types of memory within each period of the year, we used learning indexes: Tr21-30, Ts1, and Ts2-6 (Figure 6).

Repeated measures ANOVAs indicated no significant differences between the indexes for the FEB-MAR period, $F = 1.239$, $p = 0.3029$; APRIL-MAY period, $F = 0.7795$, $p = 0.4634$; JUN-JUL period, $F = 0.9107$, $p = 0.4124$; and DEC-JAN period, $F = 0.6905$, $p = 0.5087$. A Friedman test indicated no significant differences between the indexes for the AUG-SEPT period, Chi Square = 0.4286, $p = 0.8071$. Finally, a Friedman test indicated significant differences between the indexes for the OCT-NOV period, Chi Square = 13.86, $p = 0.001$. A pairwise comparison using Dunn's test indicated that Tr21-30 index scores were observed to be significantly higher than Ts1 scores, $p = 0.001$; Tr21-30 is significantly higher than Ts2-6, $p = 0.0245$; and Ts1 is not significantly different than Ts2-6, $p > 0.9999$.

The learning indexes Tr21-30, Ts1, and Ts2-6 show no significant differences within periods of the year, except for the OCT-NOV period. During OCT-NOV, the Tr21-30 index that expresses short-term memory retention capability is significantly higher than the indexes that account for the long-term memory types: CSM and SM. This suggests a disposition to use or store information different from the observed in the other periods of the year, where the indexes within periods are not significantly different. This indicates a short-term memory retention favoring tendency over the long-term memory retention.

DISCUSSION

Sensorimotor parameters exhibit annual fluctuations attributed to circannual rhythms

Both Ea and FRV undergo cyclical changes through the year.

To elucidate the factors contributing to these observed variations in sensorimotor parameters, we investigated their correlation with annual changes in physiological and environmental parameters previously associated with similar changes observed in other species. The cognitive demands of the reproductive season significantly impact memory retention abilities of deer mice, voles, and songbirds.^{18–20} The gonadal index (ratio of gonad weight to total body weight) serves as an indicator of reproductive effort and is highest during OCT-NOV for both female and male *Neohelice granulata* crabs.²¹

We assessed the relationship between Ea and the gonadal index using simple linear regressions between male Ea and the gonadal indexes of females and males (data adapted from: Lopez Greco and Rodriguez, 1999). Male crab Ea shows a correlation with female gonadal index but not with the male gonadal index (Figure S1). This suggests that interactions with conspecific individuals may influence this parameter and that crab behavioral strategies are influenced by the reproductive cycle, akin to findings in voles by Rice et al., 2019.¹⁹

The FRV varies throughout the year, exhibiting a significant decline during the colder periods (southern hemisphere). A similar result contrasting first reactions to VDS between spring-summer (October-May) and autumn/winter (April-September) was previously noted by Sztarker and Tomsic in 2008.²² These researchers also reported analogous changes in neuronal activity of VDS-responsive neurons.

Furthermore, a linear regression indicates a connection between response intensity to the VDS and the minimal water temperature of the capture location (Figure S2), suggesting a strong influence of the physical environment on this parameter. The correlation of FRV with minimal water temperature further suggests that conditions at the capture site exert an enduring impact on behavior, outweighing the influence of stable laboratory conditions prior to experimentation. This positive correlation between escape response and temperature aligns with the reported effect of temperature in neural and sensory performance, as well as escape responses in *Neohelice* and other species.^{22,23}

The alterations in sensorimotor parameters may directly impact memory retention evaluation or serve as indicators of memory retention predisposition. To mitigate the impact of escape response differences between periods, we employed retention indexes that normalize to the FRV, facilitating the evaluation of changes in memory retention capabilities over the course of the year. Both Ea and response intensity to a standardized danger significantly fluctuate between different periods of the year, underscoring the influence of circannual rhythms on these behaviors.

Memory retention parameters change during the year

A significant difference between CT and TR groups for the three parameters evaluated Tr21-30 sum (short-term memory), Ts1 (Long-term CSM), and Ts2-6 sum (long-term SM) indicates memory retention of a strategy shift in the escape response.

Short-term memory (Tr21-30 sum) indicated by significant differences between CT and TR groups is observed in all periods of the year except for APRIL-MAY period, as graphed in Figure 3A. Long-term CSM (Ts1) significant differences between CT and TR groups are observed in all periods of the year indicating a long-term CSM retention, except for OCT-NOV (Figure 4A). Long-term SM (Ts2-6 sum) shows significant differences between CT and TR groups in all periods of the year (Figure 5A), indicating a long-term SM retention. Memory retention of this strategy change is detected in all periods of the year for long-term SM, and in all periods except one in short-term memory and long-term SM. This indicates that a relevant comparison is how memory retention capabilities for each type of memory vary along the year. To address this subject, we evaluated changes between periods of the year for each of the memory retention indexes. Short-term memory retention index comparisons between periods indicate differences between OCT-NOV and FEB-MAR, APRIL-MAY, and DEC-JAN, and also between APRIL-MAY and AUG-SEPT. The short-term memory retention index is minimal in APRIL-MAY and maximal in OCT-NOV, indicating that the use of information related to the short-term escape shift is not evident in APRIL-MAY but is maximal in OCT-NOV (Figure 3B). The long-term CSM retention index shows significant differences between OCT-NOV and JUNE-JUL, AUG-SEPT, and DEC-JAN. In this case OCT-NOV being the period with the lowest index and DEC-JAN the period with the highest CSM index indicate, respectively, when the information relative to a context-dependent escape response shift after a long-term retention period was not utilized, and when this memory was maximally expressed. On the other hand, the long-term SM presents no significant index differences between periods, indicating that the use of escape strategy shift information during that part of testing is similar between periods (Figure 5B).

The differences in short-term memory and long-term CSM between periods suggest that the mnemonic capabilities of the crab are influenced by circannual rhythms. The circannual rhythms in behavior are interpreted as processes that allow an organism to efficiently utilize favorable conditions and avoid conditions that are unfavorable,¹³ suggesting that these capability changes respond to period-specific cognitive demands.

In both cases when short-term memory and long-term CSM show no retention, the animals show retention of the other two memory types. During APRIL-MAY, when there is no STM retention, CSM and SM have significant retentions, and during OCT-NOV, when there is no CSM retention, both STM and SM have significant retentions. This suggests that the information needed for the strategy change in escape response is acquired in all periods, and that the cognitive changes observed may respond to a modulation during retrieval. The retrieval process has been reported as an integration point for internal state and retained information, as well as a modulation of retention for crabs and humans.^{24,25}

Short-term and long-term information use indicate a trade-off between memory types

During two periods of the year no retention is observed for at least one type of memory; APRIL-MAY does not show STM retention and OCT-NOV has no CSM retention. STM, CSM, and SM memories retention capabilities are compared using the retention indexes within the periods of the year. Five of the six periods evaluated show no significant differences between STM, CSM, and SM indexes, including APRIL-MAY that showed no STM retention.

Unlike all other periods of the year, during OCT-NOV, STM is significantly higher than CSM and SM (Figure 6). During this period short-term memory retention reaches its maximum and long-term CSM and SM at its minimums. This difference in short- and long-term information use suggests a trade-off between both types of memories.

According to Wagner's theory of memory,²⁶ short- and long-term habituation is caused by different processes that sometimes oppose each other. The predicted competition between these processes is because the presentation of the stimuli, separated by short periods, favors short-term habituation at the expense of long-term retention while the spaced presentation of stimuli favors long-term retention at the

expense of short-term habituation. Since the interval between the VDS in this work is the same for all the periods, it is possible that what changes is the predisposition of the animals to reduce their response by changing the STM-LTM relationship toward a higher STM at the expense of a lower LTM.

A trade-off between memories is a feature already reported for other memories that also differ in their duration. Using a pharmacological approach, Chen et al., 2021, demonstrate in humans that long-term and working memory are mutually antagonistic.²⁷ In *Drosophila*, where two separate forms of memory can be generated experimentally, anesthesia-resistant memory and long-term memory have been found to be antagonistic at the functional level.^{28,29} Also, a trade-off between short- and long-term memory types has been hypothesized in *Bombus* where short-term memory performance in the laboratory predicts foraging efficiency in the wild only during the rich spring environment, indicating that an enhanced short-term memory helps to efficiently solve rapidly iterating cognitive challenges.³⁰

The OCT-NOV period when this memory trade-off is evident overlaps with the reproductive season of *Neohelice granulata*.²¹

Cognitive abilities such as learning and memory are important for survival and reproduction,³¹ and evidence from different species indicates that memory retention capabilities change during mating and reproduction season when many species are faced with complex and competitive sexual environments. In humans, males (but not females) displayed enhanced memory for details of a story when exposed to mating cues, indicating that the heightened displays of declarative memory reflect an enhancement in retrieval rather than in encoding.²⁵ During breeding male deer mice are better than females at acquisition of spatial learning.¹⁸

The coincidence in reproductive effort and a significant shift in memory retention from similar short- and long-term memory capabilities to an enhanced short-term memory and a diminished long-term memory in the OCT-NOV period, may represent a cognitive adaptation to the competitive sexual environment of the mating season. Both a rich foraging environment and a sexual competitive environment poses cognitive challenges where efficiently solving the present multiple options in short succession, could benefit from an enhanced short-term memory.

This is the first report of memory retention capability changes during the year in *Neohelice granulata*. Also, this is the first report of cognitive capability changes that are expressed as a trade-off between short- and long-term memory, which suggest that a shift to an enhanced short- at expenses of long-term use of information may represent an advantage to navigate a cognitive demanding social environment more efficiently.

Limitations of the study

Although, a shift in memory retention is evident during the onset of the reproductive season from long-term memory to short-term memory, several aspects of how this shift is achieved and which signals trigger the observed change are still questions.

Speculating in this respect, *Neohelice granulata* receptive females liberate ecdysone, a putative pheromone, to attract males.³² Also, ecdysone in *Drosophila* is critical for the formation of stable long-term memories.³³ These two facts indicate that ecdysone is an interesting candidate to study as a possible modulator of the trade-off between short- and long-term memories.

Furthermore, we have no direct indication of which behavioral aspect of the reproductive environment may benefit from this change in information use. A neuroethological approach to this last subject may shed light to the cognitive aspects of brachyuran reproduction.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.108161>.

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AUTHOR CONTRIBUTIONS

Conceptualization, F.R.; Methodology, F.R.; Investigation, R.A. and F.R.; Writing – Original Draft, F.R.; Writing – Review & Editing, F.R.; Funding Acquisition, F.R.; Resources, F.R.; Supervision, F.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the authors used GPT3.5 to improve the clarity of some sentences. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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 36. Water Temperature in Punta Rasa. <https://seatemperature.info/punta-rasa-water-temperature.html>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
<i>Neohelice granulata</i> male crabs	Captured in the rias of San Clemente del Tuyú.	Taxonomy ID: 53323
Software and algorithms		
GraphPad Prism 8.0.1	https://www.graphpad.com/	SciCrunch Registry (RRID: SCR_000306)
Other		
Marine water	Red Sea fish pharm	#10031_v20a

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ramiro Freudenthal (ramirofreudenthal@gmail.com).

Materials availability

All male *Neohelice granulata* crabs used in this article were captured from 400 m stretch of shoreline, in the coordinates included in the table, as other environments and changes in latitude may affect the results presented.

Data and code availability

Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

Data

All data reported in this paper will be shared by the [lead contact](#) upon request.

Code

This paper does not report original code.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

Adult male intertidal crabs (*Neohelice granulata*, formerly *Chasmagnathus granulatus*) were collected from water <1 m deep in narrow coastal inlets (rias) of San Clemente del Tuyú, Buenos Aires Province, Argentina. Only animals measuring 2.7–3.0 cm across the carapace and weighing ~17 g were selected to perform experiments and transported to the laboratory. Crabs were housed in plastic tanks (35 × 48 × 27 cm) filled to a depth of 2 cm with diluted marine water (Red Sea fish pharm) with a salinity of 1.0%–1.4% and a pH of 7.4–7.6. The water was changed, and the tank sanitized every 2 days. The housing room was maintained on a 12-h light–dark cycle (lights on from 07:00–19:00 h) and temperature between 22°C and 24°C. Experiments are performed after 48 h of arrival for the crabs to acclimatize and only in the first 7 days in the laboratory. Only males are used to avoid disrupting the natural population or crabs since females carry the fecundated eggs in the first stages of development and capturing them might affect the size of the population. Another factor considered is that a natural population has many sources of variability and selecting male animals restricts variables such as size range. The reported research was conducted in accordance with the local regulations for the care and use of laboratory animals (Experimental protocol certificate No. 71). All experiments were done in accordance with local regulations to minimize animal suffering and the number of animals used.

METHOD DETAILS

Behavioral apparatus

When the crabs are presented with a rectangular screen passing overhead (visual danger stimulus, VDS), the animals respond with a moving reaction to escape. This flight reaction decreases over various trials.³⁴ The crabs are placed in the actometer described elsewhere⁵ where the escape response of the animals is transduced to electrical signals integrated by a computer. The actometers are placed in an array of 40 in a single room with controlled environment in a manner that each run of an experiment has a maximum of 40 animals.³⁵ A typical experiment consists of several runs ranging from 2 to 4 runs. For the present work analyzing behavioral and mnemonic traits along the year the unit is the median response of animal groups that were in the same run (except for the example in [Figures 1 and 2A](#)), so each point in a graph consists of a range of animals between 8 and 16 crabs.

Experimental procedure

In all the experiments, crabs were distributed into two groups, consisting of a trained group (TR) and a control group (CT). The experimental procedure for TR animals could be summarized as follows. Animals were placed in the actometers, and after a 10-min period of adaptation the animals were presented with a pre-training trial (PT), the result of this first presentation of the VDS allows, first to eliminate animals with a response lower than 200 (arbitrary units) and second, to define two groups of animals with equivalent response to the VDS to be CT and TR groups. Immediately after this group assignment we proceed to a training session consisting of 30 trials given with an intertrial interval (ITI) of 100 s. Each trial lasted 5 s and consisted of passing the screen four times over the actometer and recording the response of the crab throughout the trial time. A testing session consisting of 6 trials where both CT and TR animals are presented with VDS, was performed at 24 or 48 h. During the interval between training and testing, the crabs were individually lodged in rest containers covered with water to a depth of 0.5 cm and kept inside dimly lit drawers.

The procedure for a CT was the same as that of TR, except that the animals of this group stayed in the actometers during the entire training session without being presented to the VDS. Retention for long-term habituation is operationally defined as the significant difference between CT and TR in a testing session.

Parameters evaluated

The approach to evaluate the animal responses in the periods described here was already described in Hepp et al., 2016.⁹

Exploratory activity

The exploratory activity is estimated as the median of the 30 trials, for the two-month period of CT group crabs exploring the actometer container during the training session, so there is one set of data for every run of the training sessions.

First presentation of the visual danger stimulus

The response to the visual danger stimulus is evaluated at the first exposure of all the animals during the pre-training trial (Pt).

Escape strategy shift during training

Changes in the escape strategy of the animals during the training session are represented by integrating the 10 last trials of the session (Tr21-30). During these last 10 trials the animals from the training group typically show significantly lower activity than the control groups that keep exploring the container. This difference between the control group and the trained group is interpreted as short-term memory being expressed during the training session and to be part of the learning curve.

Long-term memory retention

Long-term memory is evaluated at the testing session 24 or 48 Hours after training, with 6 presentations of the VDS. Two different parameters are evaluated, representing different components of the Long-term memory retention. The first parameter, is the animals median response to the first presentation of the visual danger stimulus during the testing session (Ts1) and significant differences between CT and TR groups interpreted as a context signal memory (CSM), and the second, the median of the integrated escape responses of trials 2 to 6 of testing session (Ts2-6) in which the differences between CT and TR groups is interpreted as retention of signal memory (SM), as described in Maldonado et al., 1997.⁶

To compare how the animals incorporate the above-described parameters between the 6 periods of the year studied, we will use the TR-CT difference with an index that relativizes the parameter (Tr21-30, Ts1 and Ts2-6) to the level of the first response in that period.

$$\Delta TR - CT \text{ Parameter } (P) \text{ index} = \frac{(P_{t_{TR}} - p_{TR})}{(P_{t_{TR}} + p_{TR})} - \frac{(P_{t_{CT}} - p_{CT})}{(P_{t_{CT}} + p_{CT})}$$

Neohelice gonadal indexes used to plot against Ea (Figure S1), are rearranged from Lopez Greco and Rodriguez, 1999 monthly data.²¹

Monthly water minimal temperatures plotted against FRV (Figure S2), are calculated based on the data over 10 years from <https://seatemperature.info/punta-rasa-water-temperature.html>.³⁶

QUANTIFICATION AND STATISTICAL ANALYSIS

Animals that responded to the pre-training trial with a response lower than 200 (arbitrary units) were eliminated from the experiments, as this low response (10 times lower than the average) is too low to evaluate a change in the escape. Animals with less than 200 (arbitrary units) are usually less than 10%.

Statistical tests used, exact value of n and what n represents for each experiment can be found in the Figure legends and results.

To group similar responses and to increase the n, the parameters were grouped in periods of two months.

Normality of data was evaluated with Shapiro-Wilk test.

To evaluate for significance of Ea between periods of the year we used a non-parametric repeated measures Friedman test, as the periods data were not normal.

To evaluate the annual variation of the first presentation to the VDS during the pre-training presentation, we used a Brown-Forsythe ANOVA test as data was normal, but there were significant differences in standard deviations of the periods of the year.

To evaluate the changes in the CT and TR group pairs for each experimental date in the 6 annual periods evaluated, we first used paired t tests when data was normal, and independent Wilcoxon matched-pairs signed rank tests when not (Figures 3A, 4A and 5A).

To contrast learning indexes, Tr21–30, Ts1 and Ts2-6 between periods of the year we used repeated measures ANOVA when the data was parametric and Friedman test when not (Figures 3B, 4B and 5B). To contrast learning indexes, Tr21–30, Ts1 and Ts2-6 within each period of the year we used repeated measures ANOVA when the data was parametric and Friedman test when not (Figure 6). Pearson correlation coefficients were performed to evaluate the relationship between variables in Figures S1 and S2, linear regression departure from zero is also informed. GraphPad 8 statistical software was used.