ABSTRACT

There is growing interest in managing cow and calf together for a prolonged period, but concerns remain about how best to wean and separate the cow and calf to minimize stress. One approach is to progressively reduce suckling opportunity over time, as in nature. There is also interest in part-time contact (suckling for part of the day) to improve milk yield for the farmer and potentially reduce stress at weaning and separation. The primary objective of this study was to compare the behavioral responses of dairy cows to gradual or abrupt weaning and separation, when managed either full- or part-time with their calves; a secondary focus was the vocal responses of calves under these management conditions. In a $3 \times 2$ factorial design ($n = 14/\text{treatment}$), dairy cows and their calves ($n = 84$ in 7 blocks of 12 cow-calf pairs) were assigned to one of 3 dam-contact treatments at birth: (1) full-time contact between the dam and calf, apart from milking times (total 23 h/d) (2) part-time contact between the dam and calf, between morning and afternoon milking only (total 10 h/d); (3) no-contact, where the dam and calf were separated after leaving the maternity pen and had no further contact. At wk 8, one of 2 weaning treatments were assigned: (1) gradual weaning by reduced contact time (50% then 25% of original dam-contact time in wk 8 and wk 9, respectively), or gradually reduced milk allowance for no-contact calves (50% then 25% of estimated 12 L milk intake in wk 8 and wk 9, respectively) until complete milk removal and dam-calf separation at wk 10; (2) abrupt weaning where milk removal occurred simultaneously with dam-calf separation at wk 10, or only milk removal for no-contact calves. Overall, part-time contact did not reduce weaning and separation distress for cows or calves, for either weaning method. Part-time cows showed reduced behavioral responses to separation (greater lying time and less searching behavior), especially on the day of and 24 h after separation, but they showed a similarly strong vocal response to separation as full-time cows. Part-time calves high-pitch vocalized substantially more than full-time calves at 24 h after separation. Furthermore, gradual weaning by reducing contact time did not seem to better prepare cows or calves for complete milk removal and separation; most behavioral and vocal responses occurred on the day of separation for gradual-wean cows and calves, but 24 h later, the reverse occurred for abrupt-wean cows and there was no difference between gradual and abrupt calves. Our results suggest that part-time contact and gradual weaning conditions likely resulted in hunger and expectation for reunion, which together may have exaggerated behavioral responses at separation. Temporary daily separations may not have increased independence of cow and calf, and the gradual weaning method here may not have reduced milk intake by calves. These elements are important criteria to facilitate the weaning process, so future work should explore ways to gradually reduce milk intake and promote social independence of cow and calf to minimize weaning distress.

Keywords: maternal contact, nursing, fence-line, animal welfare, stress

INTRODUCTION

Dairy calves are commonly separated from the mother soon after birth; this long-standing practice is argued to best protect the health of cow and calf, maximizes the harvest of saleable milk, and reduces acute stress responses to separation when done early (Beaver et al., 2019; Meagher et al., 2019). However, cow-calf separation is under increased scrutiny by laypeople and the scientific community, given it prevents natural behaviors such as nursing and bonding between the mother and her calf (Busch et al., 2017; Cardoso et al., 2017; Placzek et al., 2020; Sirovica et al., 2022).

Among farmers, there is increased interest in providing cow-calf contact, with ethical and practical consid-
erations being the biggest motivators for moving away from immediate cow-calf separation (Bertelsen and Vaarst, 2023). However, farmers often cite significant concerns and barriers to adoption of prolonged cow-calf contact systems (Neave et al., 2021; Hansen et al., 2023). One of these concerns is that later separation of cow and calf (beyond 4 d after birth) results in strong behavioral stress responses by cow and calf, including vocalizations, increased activity and searching behaviors in attempts to reunite (e.g., Veissier and Le Neindre, 1989; Weary and Chua, 2000; Flower and Weary, 2001a). These negative behavioral responses are indicators of distress arising from 2 competing processes during weaning in cow-calf contact systems: weaning from milk (i.e., transition for the calf from milk onto solid feed diet) and weaning from the dam (i.e., transition to no dam-calf contact, sometimes referred to as ‘debonding’; Wenker et al., 2022). Although weaning methods attempt to separate these 2 processes, for instance by using a nose flap or a fence-line to first prevent milk access (Johnsen et al., 2015; Wenker et al., 2023), both weaning from milk and dam occur much earlier than it would under natural or semi-natural conditions (Whalin et al., 2021). To date, there is little scientific guidance on how best to wean dairy cows and calves after prolonged contact; this knowledge is crucial given that farmers cited weaning and separation stress as their primary reason for discontinuing cow-calf contact systems (Hansen et al., 2023).

A few studies in dairy cattle have investigated use of a fence-line weaning and separation approach, which reduced the vocal response of 8- or 10-week-old calves compared with abrupt separation (Johnsen et al., 2015a; Bertelsen and Jensen, 2023). However, fence-line separation did not reduce the cows’ response unless the calf was less nutritionally dependent on the cow (because of supplementary milk access; Johnsen et al., 2018), suggesting that a gradual reduction in opportunity to nurse (to facilitate the calf’s transition toward nutritional independence of the cow) may reduce the cow’s response to separation. This approach also more closely mimics the weaning process under natural conditions where the cow progressively rejects the calf’s attempts to suckle (Reinhardt and Reinhardt, 1981). From the calf’s perspective, fence-line separation (Johnsen et al., 2015a; Bertelsen and Jensen, 2023) or insertion of a nose flap (Wenker et al., 2021) imposes an abrupt removal of milk which can contribute to weaning stress (Weary et al., 2008); when calves are consuming high milk allowances, milk should be gradually removed to encourage a transition to solid feed before complete removal of milk (Khan et al., 2016). Thus, we designed a gradual weaning method that progressively reduced daily contact between dam and calf over 2 weeks using a fence-line; this was also expected to reduce nursing time and therefore milk intake.

Farmers also raise concerns about loss of saleable milk to suckling calves (Barth, 2020), which is viewed as an economic loss despite high consumption of milk from the dam contributing to high growth rates of calves (Khan et al., 2011). Reducing the amount of contact (and suckling) time between cow and calf, such as only during the day or night, may help to increase bulk tank yield, while still allowing maternal bonding (Johnsen et al., 2015c) and high weight gains in calves (Veissier et al., 2013). Furthermore, a part-time contact system may better prepare cows and calves for weaning and separation due to familiarity with periods of separation (as suggested by Veissier et al., 2013). No study to date has examined if part-time cow-calf contact with suckling reduces the negative behavioral responses to weaning and separation in cows or calves, nor if these responses can be further reduced with reduction in contact time.

The overall objective of this study was to compare the behavioral responses of dairy cows and calves to either gradual or abrupt weaning and separation when managed in full- or part-time cow-calf contact systems. The primary focus of our study was the cow’s perspective under these management conditions, with a secondary focus being the calf. Our key outcome measure of weaning and separation distress was high and low pitch vocalizations. High-pitch vocalizations are frequent upon separation, thought to reflect attempts to reunite when visual contact is lost (Padilla de la Torre et al., 2015) and to reflect hunger (Johnsen et al., 2018), while low-pitch vocalizations in the context of separation or isolation may indicate a passive coping mechanism (Siebert et al., 2011). We predicted that our gradual weaning method involving progressive reduction of daily cow-calf contact (first 50% then 25% of original contact time in the first and second weeks, respectively) would result in reduced behavioral and vocal responses to permanent separation, compared with cows and calves that were abruptly weaned and separated; we expected these responses would be further reduced in part-time compared with full-time contact systems.

**MATERIALS AND METHODS**

This study was conducted from September 2021 to August 2022 at the Danish Cattle Research Centre, Aarhus University (Tjele, Denmark). All animal procedures were approved by the Danish Animal Experiments Inspectorate (Permit No. 2021–15–0201–00989) in accordance with the Danish Ministry of Environment and Food Act No. 474 (May 15, 2014).
Experimental design and animal enrolment

Eighty-four Danish Holstein dairy cows and their calves were enrolled in 7 blocks of 12 cows each. Each block consisted of a 2-wk enrolment period followed by a 10-wk experimental treatment period. All cows calved in an individual maternity pen where they remained with their calf for approximately 48 h (range: 42 to 66 h). To be eligible for enrolment, the cow and calf had to be healthy, have no twin births or calving complications, and the calf had to be able to suckle from the cow without assistance within 48 h. Cows and their calves were then moved from the maternity pen at 1000 h or 1300 h (depending on calving time) to one of 3 dam-contact treatment pens: (1) full-time contact between the dam and calf, apart from milking times (total 23 h/d of cow-calf contact); (2) part-time contact between the dam and calf, between morning and afternoon milking only (total 10 h/d of cow-calf contact); (3) no-contact, where the dam and calf were separated after leaving the maternity pen and had no further contact (no-contact cows were only observed for 24 h daily lying time as the control group). Only the calves in the no-contact treatment were the focus of the current study. Assignment to dam-contact treatment occurred in pairs (i.e., 2 cow-calf pairs to avoid stress of entering a pen alone), and order of treatment assignment for a pair was pre-determined for each block on a rotational basis (e.g., Block 1: first pair born was assigned to part-time, second pair to full-time, third pair to no-contact, and repeated for the remaining 3 pairs to fill the block; Block 2 order: full-time, no-contact, part-time; Block 3 order: no-contact, part-time, full-time; and so on until 7 blocks were filled). Each treatment pen within a block were balanced for 2 primiparous and 2 multiparous cows whenever possible; however, one primiparous and 3 multiparous cows were in 3 of 7 full-time pens, and in 2 of 7 part-time pens. Final distribution was similar between primiparous (n = 11, n = 12) and multiparous cows (n = 17, n = 16) in the full-time and part-time treatments, respectively. Average lactation of multiparous cows in full- and part-time treatments was 2.3 ± 0.6 (range 2 to 4) and 2.7 ± 0.9 (range 2 to 5) lactations, respectively. Once all 12 cows were enrolled in the block, the experimental period began (nominally referred to as wk 1 to 10). Calf age at the start of the experiment (wk 1) was on average 10 d (mean ± SD; full-time: 8.8 ± 6.9 d; part-time: 10.7 ± 5.9 d; no-contact: 10.5 ± 5.2 d), and the age gap between the oldest and youngest calf in a pen of 4 calves was on average 11 d (mean ± SD; full-time: 12.0 ± 6.4 d; part-time: 10.7 ± 2.2; no-contact: 10.1 ± 4.6 d). Distribution of female and male calves across dam-contact treatments was not randomized as part of the experimental design, but the final distribution was similar (full-time: 17 female, 10 male; part-time: 13 female, 13 male; no-contact: 14 female, 12 male).

At wk 8 of the experimental period, cow-calf pairs were pseudo-randomly assigned to one of 2 weaning treatments, ensuring a balance of primiparous and multiparous cows: (1) gradual weaning over 2 weeks by reducing contact time, until complete milk removal and separation from the dam at wk 10; (2) abrupt weaning, where complete milk removal occurs simultaneously with complete separation from the dam at wk 10. For no-contact calves, the above treatments were applied with respect to milk removal only (since they are already separated from the dam). In the dam-contact treatments, distribution of primiparous and multiparous cows across weaning treatments was balanced (gradual wean: n = 16 primiparous, n = 12 multiparous; abrupt wean: n = 11 primiparous, n = 17 multiparous).

Thus, the experimental design resulted in a 3 × 2 factorial design with 14 cow-calf pairs assigned to each combination of housing and weaning treatment. This sample size was determined from a power analysis with α = 0.05 and power = 0.80, using the estimated standard deviation of vocalization frequency (our primary outcome measure) reported in Johnsen et al. (2015) and an expected 20% difference in vocalizations between treatments.

Dam-contact treatments

Full-time and part-time cows and their calves were housed in a dedicated barn in straw-bedded pens (7.5 × 9 m) containing 4 cows and 4 calves (see Figure 1 for pen placement in the barn; treatments were balanced across pen locations). In each pen (Figure 2a), calves had exclusive access to a small and large creep area (3 × 3 m and 1.5 × 1.5 m) at the back corners of the main pen; the location of these creeps alternated between replicate pens. Each creep contained ad libitum concentrate from a bowl and hay from a rack, and the larger calf creep area contained a self-filling water bowl. The creeps were constructed of fences with tubular metal bars that permitted full visual and some physical contact with the cow through the bars, but nursing was not possible through the bars (when restricted to this area during weaning). The sides of the main pen were constructed of the same type of fence, but were fixed with solid plastic siding to prevent visual contact with animals in the adjacent pen. At the front of the main pen, cows and calves had access to 2 feed troughs (each 2 × 0.75 m) with ad libitum total mixed ration (TMR; approximately 50:50 concentrate to roughage ratio) that was replenished twice daily at 0800 and 2000 h (refusals removed at 0800). Cows also had access to...
2 rotating grooming brushes and 2 self-filling water bowls, mounted on opposite sides of the pen. Straw bedding was added daily and completely cleaned out approximately every 4 wk. Calves received all of their milk by nursing from their dam or other cows in the pen.

Full-time and part-time cows were milked twice daily at 0500 and 1530 h in a double 12 parallel milking parlor. After each afternoon milking, part-time cows were redirected via sorting gates to a pen in a separate barn without visual or auditory contact with their calves; this pen held 8 cows (4 part-time cows each from block \( n \) and block \( n+1 \)) (Figure 2b). This pen contained 14 lying stalls equipped with mattresses and topped with sawdust daily, and TMR (as described above) for \textit{ad libitum} intake was delivered at a feed bunk with headlocks, replenished at 2000 h. After morning milking, part-time cows returned to their home pen to be reunited with their calves. Full-time cows always returned to their home pen after each milking.

No-contact calves were housed in pens adjacent to the full-time and part-time cows and calves (Figure 2c), with auditory contact to the full-time and part-time cows, but not their dams (who were housed in a separate barn, described below). Calves had visual contact to cows and calves in the pen placed directly opposite their pens, but not the other pens (see Figure 1 for pen placement in the barn). Calves initially were housed in straw-bedded pens \((1.5 \times 1.5 \text{ m})\) individually for 7 d, then paired with neighboring calves until a pen of 4 calves was formed \((3 \times 3 \text{ m})\). The front of this pen contained 2 bowls each of concentrate and TMR that were refreshed daily at 0630 h. Calves also had access to hay from a rack that was shared by 2 pens of calves. In the first 7 d, calves were fed warm milk beginning at 6 L/d and increasing to 8 L/d in 2 daily feedings. After grouping, calves were fed \textit{ad libitum} warm milk from twice daily until satiation (milk was topped up part-way through feeding) at 0630 and 1700 h, until wk 8 when milk allowance was reduced (see Weaning section below). Milk was always offered from a single teat bucket (one plastic bucket with one teat per calf (Peach Teat, Skellerup Industries, Ltd., Christchurch, New Zealand). No-contact cows were housed in a pen in a separate barn from the full-time and part-time cows and calves, but were not involved in data collection for the present study and are not discussed further.

Figure 1. Layout of dam-contact and no-contact calf pens in the barn. Full-time and part-time contact treatment pens for a given block \( n \) were always on opposite sides of the barn, and adjacent to the next block \( n+1 \). Treatment side alternated between blocks. At separation, dam-contact calves (full-time and part-time) were housed in adjacent pens next to the no-contact calves, maintaining treatment group. Shaded gray rectangles inside the full-time and part-time pens indicate the calf creep areas, and outside indicate the feed troughs.
Figure 2. Housing pens for full-time, part-time and no-contact treatments. (A) Pen for full-time cows and calves (during 23 h/d, excluding milking times), and for part-time cows and calves (during daytime only, from 0530 to 1530 h). Section 1 houses both cows and calves and permits full interaction. Section 2 is a small calf creep with one entrance containing hay and concentrate only for calves. Section 3 is a large calf creep with 2 entrances containing hay, concentrate and water bowl only for calves. Cows could place their head through the entrances, or over the fences of the calf creeps, but could not fully enter. (B) Separation pen for part-time cows during nighttime (1530 h to 0500 h), containing 14 lying stalls bedded with mattresses and sawdust. (C) Pens for no-contact calves (Section 1 during wk 1–7; pen divided for wk 8–9 as pictured in Section 2; pen returned to group of 4 for wk 10) and pens for full-time and part-time calves at separation in wk 10 (Section 3 and 4, with one treatment group per pen). Hay, concentrate and water bowls are in each pen division.
Figure 3. Cow-calf contact schedule outlining periods of cow-calf contact (colored), temporary separation (white), and complete separation (black), for each weaning (gradual, abrupt) and dam-contact treatment (full-time, part-time) (n = 14/treatment). Week 1 to 7: full-time (dark color) and part-time (light color) treatments had cow-calf contact for either 23 h/d or 10 h/d, respectively. Week 8 and 9: gradual-wean treatments (orange) had dam-contact time reduced to 50% of original time in wk 8, then a further reduction to 25% of original time in wk 9; abrupt-wean treatment (blue) experienced no change in dam-contact time. Week 10: permanent separation (black) occurred at 1100h on the day of separation; abrupt-wean treatments (blue) had dam-contact in the hours before separation, but gradual-wean treatments did not. Observation start times are indicated by yellow circle.

Weaning treatments

Figure 3 provides a summary of the gradual and abrupt weaning schedules for full-time and part-time dam-contact treatments. Two days before weaning treatments were initiated at wk 8, the pens of the full-time and part-time dam-contact treatments were divided in half to create 2 weaning treatments, with 2 cows-calf pairs on either side of the fence; the gradual weaning treatment was always on the side with the large calf creep to accommodate restricting calves to this area (Figure 4a). This pen division was done before weaning began so that cows and calves could habituate to the smaller pen and group size. The gradual weaning treatment involved systematically reducing the amount of contact time and thus the time the calves could nurse from the dam, theoretically resulting in reduced milk intake and gradual physical separation from the dam. Outside of the permitted contact times, research staff restricted calves to the large calf creep by blocking the exits with a temporary fence that allowed visual and limited physical contact with the dam, but not nursing (Figure 4b). During wk 8, time was reduced to approximately 50% of the original allotted dam-contact time (full-time dam-contact: only 9.5 h/d, between 1100 and 2100 h, excluding afternoon milking; part-time dam-contact: only 4.5 h/d, between 1100 and 1530). During wk 9, time was further reduced to approximately 25% of the original allotted dam-contact time (full-time dam-contact: only 4.5 h/d, between 1100 and 1530; part-time dam-contact: only 2 h/d, between 1100 and 1300). The abrupt weaning treatment experienced no change in their allotted dam-contact time from wk 8 to wk 9.

No-contact calves also experienced pen division 2 d before weaning treatments began at wk 8 (see Figure 2c, Section 2). For gradual weaning, milk allowance was restricted to approximately 50% of original allowance.
(from ad libitum estimated at 12 L/d to 6 L/d, in 2 feedings) during wk 8, then restricted to 25% of the original allowance (to 3 L/d, in 2 feedings) during wk 9. The abrupt weaning treatment experienced no change in milk allowance from wk 8 to wk 9.

**Separation and milk removal period**

On Monday of wk 10, milk was completely removed from all calves. Calves with full-time and part-time dam-contact also experienced complete separation from the dam at 1100 h, when these calves were removed from their home pens and placed in a separation group pen identical to and adjacent to the no-contact calves and in the same barn as their dams; dam-contact treatment groups were maintained in adjacent separation pens (Figure 2, Section 3–4). Therefore, they had auditory contact with their dams, but no visual contact (however some visual contact was possible for 3 pens that were positioned across the alleyway from the calf group pens). For no-contact calves, the pen division was removed to form the original group of 4 calves. All calves remained in the separation pens, and dam-contact cows in their home pens, for 4 d until the experimental period ended.

**Behavioral observations**

During wk 1, each cow-calf pair was marked with one of 4 unique symbols using hair dye to facilitate identi-
fication of individual cows and calves in the treatment pen during behavioral observations. For all observations, blinding was not possible due to direct and video observations revealing treatment conditions.

**Vocalizations (cows and calves).** The number of high-pitched, low-pitched, and mixed vocalizations from each animal (see Table 1 for ethogram) were continuously recorded by direct observation for each cow and calf in the pen in a 2 h period before and after the weaning and separation periods. Observation times occurred the day before, day of, 24 h after, and 48 h after the weaning and separation interventions at wk 8 and wk 10, respectively (see Figure 3). Observations before and after weaning initiation occurred immediately after cows returned from morning milking (about 0530 to 0730 h), and observations before and after separation occurred immediately after calves were separated from the dams (1100 to 1300 h). No-contact calves (only Block 4 to 7) were observed only before and after the corresponding separation period for full-time and part-time calves (when no-contact calves experienced complete milk removal). On a given observation day, one observer was responsible for recording all vocalizations from each animal in a treatment pen (i.e., 2 observers during weaning observations for full-time and part-time pens; 5 observers during separation observations for full-time and part-time cow and calf pens, and the no-contact calf pen). Observers had a 10-min break after 55 min of observation, resulting in a total observation time of 110 min per day per animal. Observers sat on a raised surface (for cows: 2.5 m high referee chair; for no-contact and separated calves: 1.5 m high table) placed 1.5 m from the front of the pen to ensure visibility of all animals. Interobserver agreement on recording of vocalization frequency and type was achieved before performing direct observations, using a separate population of cows from another experiment exposed to a novel arena to induce vocalizations (Interobserver agreement (IOA) = 82.1, 80.3 and 76.7% agreement for total counts per cow of high-pitched, low-pitched and mixed vocalizations, respectively; Hausman et al., 2022).

**Pen behaviors (cows only).** The behaviors of cows were continuously recorded from video using Behavioral Observation Research Interactive Software (BORIS) during the same observation periods at weaning and separation interventions (see Table 1 for ethogram). Start and end times of each 2 h observation period aligned exactly with the vocalization direct observation period. Behaviors recorded before and after initial weaning intervention at wk 8 were: close to the calf creep fence, head over the calf creep fence, lying time, contact with own calf, contact with alien calf (the latter 2 were recorded for purposes of a concurrent study and not considered further). Behaviors recorded before and after separation interventions at wk 10 were: head over perimeter fence and lying time. Video recordings were obtained from cameras installed above each pen (HIKVISION DS-2CD2345FWD-I(2.8mm) DS-2CD-2345FWD-I - Pro Series, Hangzhou Hikvision Digital Technology Co., Ltd., China) using BlueIris video management software (BlueIris Security, Bozeman, MT, USA). Four observers (2 for Block 1 to 2; one for Block 3 to 6; one for Block 7) obtained inter-observer reliability (Cohen’s kappa, κW >0.90).

**Activity (cows and calves).** To monitor daily 24-h lying time as a measure of restlessness during weaning and separation, accelerometers (IceQube data loggers, Ice Robotics Ltd.) were attached to the rear leg of each cow and calf from all treatments in wk 6 to allow ample habituation to the device (validated in calves by Trénel et al., 2009; in cows by Borchers et al., 2016). Data were downloaded weekly from the accelerometers by scanning with a triggering device while attached to the animal.

**Data handling and final sample size**

One part-time calf (Block 4) was euthanized in wk 3 due to poor health and growth, and the calf’s dam was also removed from the treatment pen; the remaining 3 cow-calf pairs were all assigned to gradual wean treatment (no pen division was performed to allow sufficient space). One part-time abrupt wean calf (Block 1) was euthanized at separation (before milk removal) due to severe illness and poor growth. Two no-contact calves (Block 1 and Block 5) were euthanized before weaning due to severe illness and poor growth. The final sample sizes in the 3 × 2 factorial design were: 14 full-time cow-calf pairs in each of gradual and abrupt wean treatments; 15 and 12 part-time cow-calf pairs in gradual and abrupt wean treatments, respectively; and 13 no-contact calves in each of gradual and abrupt wean treatments, respectively.

For the 110 min observation periods, the sum count of vocalizations and total duration of each behavior were summarized for each animal, for each day relative to weaning or separation. For daily 24-h lying time, the sum of quarter-hourly recordings from each cow and calf were calculated for the day of, 24 h and 48 h after weaning initiation (wk 8, where abrupt wean served as the control comparison) and the day before, day of, 24 h and 48 h after separation (wk 10, where day before served as the control comparison). The summed interval was from 0530 h to 0530 h the next day during weaning, and from 1100 h to 1100 h the next day during separation. These intervals corresponded with the start of the 110 min observation periods at weaning and
separation, respectively. Activity data was removed for reasons of logger malfunction (n = 14 cows and n = 4 calves resulting in 0 records for all days), logger misidentification (n = 2 calves recorded as having the same logger), days with less than 24 h per day (n = 64 of 1055 records), days with 0 h recorded for both lying and standing (n = 14 records) or calf was euthanized (n = 1 cow). The final sample size was 457 records from n = 68 cows (abrupt-wean: n = 13, 12, and 10 cows; gradual-wean: n = 11, 14, and 8 cows in full-time, part-time and no-contact treatments, respectively), and 499 records from 78 calves (abrupt-wean: n = 14, 11, and 13 calves; gradual-wean: n = 14, 15, and 11 calves in full-time, part-time and no-contact treatments, respectively).

**Statistical analysis**

All statistical analyses were performed using SAS Studio (OnDemand for Academics, SAS Institute Inc.), where cow within pen and block was the experimental unit. Results are presented as least squares (LS) means and standard error (SE), or as back-transformed means and confidence limits (CL) for data requiring log10 transformations. Pairwise comparisons are performed using Tukey-Kramer adjustment for significant interactions (t statistic reported). Significance level was declared at $P \leq 0.05$, and tendencies at $P \leq 0.10$.

**Vocalizations (cows and calves).** For the weaning period, the frequency distributions of vocalizations (all types) were heavily zero weighted for both cows and calves; thus, the number of cows and calves that vocalized at least once for each vocalization type in each dam-contact (full-time or part-time) and weaning treatment (abrupt or gradual) were tallied and analyzed using a Fisher exact test (PROC FREQ), stratified by day relative to weaning initiation.

For the separation period, a mixed Poisson regression model (PROC GLIMMIX) tested whether weaning and dam-contact treatments affected the frequency of each vocalization type, modeled separately for cows and calves. Cows and calves rarely vocalized before separation, so for best model fit, the analysis was restricted to the day of, 24 and 48 h after separation (data from

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
<th>Time period recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vocalizations (cows and calves)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-pitched vocalization</td>
<td>Cow or calf making audible sound through the mouth with the mouth fully open, with inhalation between two occurrences. Resembles a [“muh”] sound. Also referred to as open-mouth vocalization.</td>
<td>Before and after weaning + separation. No-contact calves: only before and after separation.</td>
</tr>
<tr>
<td>Low-pitched vocalization</td>
<td>Cow or calf making audible sound through the mouth with the mouth closed. Resembles a [“mm”] sound. Also referred to as closed-mouth vocalization.</td>
<td>Before and after weaning + separation. No-contact calves: only before and after separation.</td>
</tr>
<tr>
<td>Mixed vocalization</td>
<td>Cow or calf making audible sound through the mouth, where the initial sound begins with the mouth closed and transitions to mouth open. Resembles a [“mm ..muh”] sound.</td>
<td>Before and after weaning + separation. No-contact calves: only before and after separation.</td>
</tr>
<tr>
<td>Pen behaviors (cows only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close to the calf creep fence</td>
<td>Two front feet and the whole part of the head are within 1.5 m of the calf creep.</td>
<td>Before and after weaning only. Gradual-wean treatments only</td>
</tr>
<tr>
<td>Head over the large calf creep fence</td>
<td>Cow’s head (any part of it) is over the calf creep fence. Exclude if cow is rubbing or licking while head is over the fence, and if nose is between bars.</td>
<td>Before and after weaning only. Gradual-wean treatments only</td>
</tr>
<tr>
<td>Contact own calf</td>
<td>Cow’s muzzle or tongue is touching any part of own calf’s head or body, while sniffing, licking. Touching is defined as we can see no visible gap between the cow’s head and the calf’s body or head.</td>
<td>Before and after weaning only</td>
</tr>
<tr>
<td>Contact alien calf</td>
<td>Cow’s muzzle or tongue is touching any part of an alien calf’s head or body, while sniffing, licking. Touching is defined as we can see no visible gap between the cow’s head and the calf’s body or head.</td>
<td>Before and after weaning only</td>
</tr>
<tr>
<td>Head over perimeter fence</td>
<td>Cow’s head (any part of it) is over the pen perimeter fence, excluding the calf creep fence and the fence over the feed trough. Exclude if cow is rubbing or licking while head is over the fence, and if nose is between bars.</td>
<td>Before and after weaning + separation</td>
</tr>
<tr>
<td>Lying down</td>
<td>Legs are not supporting the body, in any resting position. Resting either on sternum, or laterally. Head may be rested on body or surface, or be raised.</td>
<td>Before and after weaning + separation</td>
</tr>
</tbody>
</table>
before separation presented descriptively). The no-contact calves also rarely vocalized at any point of the separation period, so for best model fit, the analysis of calf vocalizations was restricted to only full-time and part-time contact treatments (no-contact calf vocalizations presented descriptively). The outcome variable was the daily vocalization count (modeled separately for high-pitched, low-pitched and mixed types), and the fixed effects were: weaning treatment (gradual; abrupt), dam-contact treatment (full-time; part-time), day after separation (day of; 24 h; 48 h), cow parity (primiparous; multiparous), calf sex (male; female), calf age, and the 2 and 3-way interactions of weaning treatment, contact treatment and day after separation. Random effects of pen and block were included, accounting for dependency among calves in the same pen and the same block; the repeated observations of day were modeled with autoregressive covariance structure. Type II sum of squares and Kenward-Roger calculation method for degrees of freedom were specified.

High- and low-pitch vocalizations in the context of weaning and separation are thought to reflect different coping mechanisms (Padilla de la Torre et al., 2015), and we suggest the sum may reflect an overall measure of distress during the process. The sum total (cumulative) vocalizations were examined for extreme outliers, separately for cows and calves (none were identified). A mixed Poisson regression model (PROC GLIMMIX) tested whether weaning and dam-contact treatments affected the cumulative frequency of vocalizations, modeled separately for cows and calves. The model was identical to that described above, excluding day after weaning or separation since data was summed over days.

**Behavior observations (cows only).** In the weaning period, only behavior of gradual-wean cows was analyzed, using the day before weaning initiation as the within-subject control. Time spent close to the calf creep fence required a log10 transformation to achieve an approximate normal distribution. A mixed regression model (PROC MIXED) tested whether dam-contact and weaning treatments affected the time spent lying and time spent with head over the perimeter fence. The models contained the same fixed and random effects as described above, with type II sum of squares, autoregressive covariance structure, and between-within degrees of freedom approximation.

**Activity (cows and calves).** All activity data from the sensors was examined for extreme outliers; one cow recorded a very high lying time of 21 h on the day after weaning, which led to skewed model residuals and was therefore removed. Separate models for cows and calves, at each of weaning and separation periods, tested whether dam-contact and weaning treatments affected daily 24-h lying time; the models were identical to those described for lying time in the 110 min observation period, except that no-contact cows were included as a control group.

## RESULTS

### Weaning period

Overall, the gradual wean treatment induced some vocal response, primarily low-pitched, in cows and calves, and induced behavioral responses in cows during the weaning initiation period. Dam-contact treatment had little effect on vocal or behavioral responses to weaning initiation.

**Vocalizations (cows and calves).** The frequency distribution of the number of cows and calves that vocalized at least once on each day relative to weaning are shown in Table 2. Compared with abrupt weaning (who experienced no change), more cows and calves responded to gradual weaning initiation by vocalizing (of any type) on the day of and 24 h after weaning initiation, and 48 h after there was still more gradual-wean calves that low-pitch vocalized (Table 2a). Within the gradual-wean treatment, dam-contact treatment did not affect the number of cows that vocalized after weaning initiation on any day (all vocalization types), but did affect the number of calves that vocalized on the day of gradual weaning (more part-time than full-time calves, all vocalization types), and 24 h later (more full-time than part-time calves, low-pitch vocalizations only) (Table 2b).

**Behavior observations (cows only).** Among the gradual-wean cows (n = 29), more responded to weaning initiation by placing their head over the calf creep fence on the day of and 24 h after weaning (18/29 and 11/29 cows, respectively; \( P < 0.02 \)), but not 48 h after (7/29...
Neave et al.: Weaning and separation of dairy cows from their calves

Table 2. Frequency distribution of the number of cows and calves that vocalized at least once on each day relative to reducing contact time by 50% for gradual weaning treatment in wk 8. (A) comparison of weaning treatments: abrupt versus gradual wean; (B) within the gradual-wean treatment, comparison of dam-contact treatments: full-time versus part-time. Results from Fisher Exact test are reported separately for each type of vocalization (high-pitched, low-pitched or mixed). Significant results are bolded ($P < 0.05$); tendencies are italicized ($0.05 < P < 0.1$); NC = not computed. Sample size: n = 26 abrupt weaned cow-calf pairs; n = 29 gradual weaned cow-calf pairs, of which: n = 14 full-time, n = 15 part-time.

(A) Abrupt versus gradual wean treatments

<table>
<thead>
<tr>
<th>Day relative to weaning</th>
<th>High-pitched vocalizations</th>
<th>Low-pitched vocalization</th>
<th>Mixed vocalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abrupt</td>
<td>Gradual</td>
<td>P value</td>
</tr>
<tr>
<td>Cows</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1</td>
<td>0</td>
<td>0.47</td>
</tr>
<tr>
<td>Day of</td>
<td>2</td>
<td>8</td>
<td>0.08</td>
</tr>
<tr>
<td>24 h</td>
<td>0</td>
<td>8</td>
<td>0.005</td>
</tr>
<tr>
<td>48 h</td>
<td>0</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Calves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td>Day of</td>
<td>0</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 h</td>
<td>0</td>
<td>10</td>
<td>0.001</td>
</tr>
<tr>
<td>48 h</td>
<td>0</td>
<td>4</td>
<td>0.12</td>
</tr>
</tbody>
</table>

(B) Within gradual wean treatment: Full-time versus part-time treatments

<table>
<thead>
<tr>
<th>Day relative to weaning</th>
<th>High-pitched vocalizations</th>
<th>Low-pitched vocalization</th>
<th>Mixed vocalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full-time</td>
<td>Part-time</td>
<td>P value</td>
</tr>
<tr>
<td>Cows</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td>Day of</td>
<td>3</td>
<td>5</td>
<td>0.68</td>
</tr>
<tr>
<td>24 h</td>
<td>5</td>
<td>3</td>
<td>0.43</td>
</tr>
<tr>
<td>48 h</td>
<td>1</td>
<td>0</td>
<td>0.48</td>
</tr>
<tr>
<td>Calves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td>Day of</td>
<td>1</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 h</td>
<td>6</td>
<td>4</td>
<td>0.44</td>
</tr>
<tr>
<td>48 h</td>
<td>2</td>
<td>2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

cows), compared with before weaning (2/29 cows), with no difference between dam-contact treatments.

Test statistics and P-values for time spent close to the calf creep fence and lying behavior during observation at weaning are reported in Table 3. Time spent close to the calf creep fence in the gradual wean treatment was similar before (15.2 min/observation, CL: 8.8 – 26.3 min/observation), day of (24.5 min/observation, CL: 14.1 – 42.2 min/observation), and 24 h after weaning (18.3 min/observation, CL: 10.6 – 31.7 min/observation), but was less by 48 h after weaning (18.3 min/observation, CL: 10.6 – 31.7 min/observation), and did not differ between dam-contact treatments (full-time: 17.7 min/observation, CL: 10.0 – 31.2 min/observation; part-time: 13.1 min/observation, CL: 7.6 – 22.6 min/observation).

Among gradual-wean cows, lying time (in the 110 min observation period at intervention) generally increased over time after weaning initiation, where lying time was lower on the day of weaning (75.1 ± 3.1 min/observation) and before weaning when nursing was possible (77.2 ± 3.1 min/observation) compared with 24 and 48 h after weaning initiation (88.9 and 92.8 ± 3.0 min/observation, respectively; $t_{1,81} > 2.8; P < 0.01$) (Table 3). Full-time cows tended to have lower lying time than part-time cows during gradual weaning (80.1 ± 2.4 vs. 86.9 ± 2.5 min/observation, respectively).

Activity (cows and calves). Gradual weaning initiation did not impact daily 24-h lying time in cows, and there was no dependency on day relative to weaning (Table 3). Full-time cows had greater daily lying time than part-time and no-contact cows (14.5 ± 0.29, 12.1 ± 0.27, 11.3 ± 0.34 h/d, respectively; $t_{1,59} > 6.1; P < 0.001$), and part-time cows tended to have greater daily lying time than no-contact cows ($t_{1,59} = 1.9; P = 0.07$).

In calves, gradual weaning initiation also did not impact the daily 24-h lying time, and there was no dependency on day relative to weaning (Table 3). Part-time calves had the lowest daily lying time (17.1 ± 0.2 h/d; $t_{1,67} > 2.8; P < 0.01$) compared with full-time (17.9 ± 0.2 h/d; $t_{1,69} = 2.8; P < 0.01$) and no-contact calves (18.3 ± 0.2 h/d; $t_{1,69} = 4.1; P < 0.001$), with no difference in daily lying time between full-time and no-contact calves.
Table 3. Model results (F and P values, with degrees of freedom in subscripts) from the Poisson mixed regression analyses testing the effects of weaning treatment (W), contact treatment (C), day (D) and their two-way interactions on vocalizations and behaviors during the weaning (wk 8) and separation periods (wk 10). Estimates and SEM are shown in Figures 5–8. Cow parity, calf sex, calf age at the time of weaning or separation were included as fixed effects, and three-way interaction of W × C × D (none significant, not reported). Significant results are bolded (P < 0.05), and tendencies are italicized (0.05 < P < 0.1). Sample size for each W × C combination were: n = 14, 14, 15, 12 cow-calf pairs, for full-time gradual wean, full-time abrupt wean, part-time gradual, and part-time abrupt wean treatments. No-contact calves (n = 13) rarely vocalized so were excluded from dam-contact analyses (results reported separately).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Weaning treatment (W)</th>
<th>Dam-contact treatment (C)</th>
<th>Day of weaning or separation (D)</th>
<th>W × C</th>
<th>W × D</th>
<th>C × D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cows</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close to the calf creep fence (min)</td>
<td>N/A ^2</td>
<td>F₁,44.7 = 0.07</td>
<td>F₁,44.6 = 0.25</td>
<td>F₂,104.8 = 28.0</td>
<td>F₁,44.5 = 0.24</td>
<td>F₂,107.3 = 28.5</td>
</tr>
<tr>
<td>Lying time in first 120 min (min)</td>
<td>N/A ^2</td>
<td>F₁,44.7 = 3.6</td>
<td>F₁,44.6 = 3.3</td>
<td>F₂,104.8 = 8.0</td>
<td>F₁,44.5 = 0.91</td>
<td>F₂,107.3 = 5.3</td>
</tr>
<tr>
<td>Daily 24-h lying time (h/d)</td>
<td>F₁,58.6 = 0.67</td>
<td>F₂,59 = 31.4</td>
<td>F₂,111 = 0.51</td>
<td>F₂,59 = 0.70</td>
<td>F₂,111 = 0.50</td>
<td>F₂,111 = 0.12</td>
</tr>
<tr>
<td><strong>Calves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily 24-h lying time (h/d)</td>
<td>F₁,69 = 0.06</td>
<td>F₂,69 = 9.2</td>
<td>F₂,111 = 0.49</td>
<td>F₂,69 = 1.0</td>
<td>F₂,111 = 0.17</td>
<td>F₂,111 = 0.31</td>
</tr>
</tbody>
</table>

**Separation and milk removal period**

<table>
<thead>
<tr>
<th>Variable</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>High-pitched vocalizations</td>
<td>F₁,44.8 = 0.36</td>
<td>F₁,44.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,44.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Low-pitched vocalizations</td>
<td>F₁,44.8 = 0.36</td>
<td>F₁,44.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,44.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Mix vocalizations</td>
<td>F₁,44.8 = 0.36</td>
<td>F₁,44.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,44.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Head over perimeter fence (min)</td>
<td>F₁,44.8 = 0.36</td>
<td>F₁,44.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,44.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Lying time in 110 min observation (min)</td>
<td>F₁,44.8 = 0.36</td>
<td>F₁,44.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,44.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Daily 24-h lying time (min)</td>
<td>F₁,44.8 = 0.36</td>
<td>F₁,44.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,44.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td>Calves</td>
<td>High-pitched vocalizations</td>
<td>F₁,48.8 = 0.36</td>
<td>F₁,48.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,48.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Low-pitched vocalizations</td>
<td>F₁,48.8 = 0.36</td>
<td>F₁,48.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,48.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Mix vocalizations</td>
<td>F₁,48.8 = 0.36</td>
<td>F₁,48.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,48.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
<tr>
<td></td>
<td>Daily 24-h lying time (min)</td>
<td>F₁,48.8 = 0.36</td>
<td>F₁,48.7 = 0.84</td>
<td>F₂,104.8 = 21.0</td>
<td>F₁,48.5 = 0.49</td>
<td>F₂,107.3 = 25.5</td>
</tr>
</tbody>
</table>

^1Analysis only performed for cows in the gradual-wean treatment, where the day before weaning served as the control.

**Vocalizations (cows and calves).** In cows, there was no interaction between weaning × dam-contact treatments, but there was an interaction of both weaning and dam-contact treatments with day of separation affecting the frequency of high-pitched (Figure 5a) and low-pitched (Figure 5b) vocalizations; there was only a weaning treatment × day of separation interaction for mix vocalizations (Figure 5c). Gradual-wean cows vocalized more (all types) than abrupt wean cows on the day of separation (t₁,180.9 > 4.3; P < 0.001), but this pattern reversed at 24 h after separation (t₁,177.9 > 2.0; P < 0.05), and abrupt wean cows still tended to vocalize more at 48 h after separation (t₁,173.9 > 1.7; P < 0.10). Dam-contact treatments were similar in frequency for all vocalization types each day, except that part-time vocalization types were increased for dam-contact treatments compared to no-contact treatments.
cows tended to high-pitch vocalize more at 48 h after separation ($t_{1.99.0} = 1.7; P < 0.10$).

In dam-contact calves, the bulk of vocalizations occurred 24 h after separation, with close to 300 vocalizations per calf in a 110 min observation period, and the vast majority of these were high-pitched vocalizations. There was no interaction between weaning × dam-contact treatments, but there was a weaning treatment × day of separation interaction affecting the frequency of all vocalization types (Figure 6a-c). Gradual-wean calves were more vocal (all types) than abrupt-wean calves on the day of separation ($t_{1.147} > 3.9; P < 0.01$), but did not differ at 24 h and 48 h after separation. Dam-contact treatment also affected calf vocalizations, depending on day of separation for high-pitched vocalizations; full-time calves tended to high-pitch vocalize more on the day of separation ($t_{1.147} = 1.9; P = 0.06$), while part-time calves high-pitched vocalized more at 24 h after separation ($t_{1.100} = 2.2; P = 0.03$; Figure 6a). Part-time calves low-pitch vocalized more than full-time calves (Figure 6b). There was a dam-contact treatment × day of separation interaction for mixed vocalizations, where part-time calves vocalized more at 48 h after separation (Figure 6c).

No-contact calves rarely vocalized during the milk removal period (corresponding to the separation period in dam-contact calves). On day of, 24 h and 48 h after milk removal, abrupt-wean calves averaged (mean ± SD) $1.1 ± 2.6, 0.0 ± 0.0, and 0.14 ± 0.38$ vocalizations/observation, respectively, and gradual-wean calves averaged $1.0 ± 1.9, 0.0 ± 0.0, and 0.13 ± 0.35$ vocalizations/observation, respectively, across all vocalization types.

**Behavior observations (cows only).** Time spent with head over the perimeter fence had 2-way interactions of weaning and dam-contact treatments with day of separation (Figure 7a). Both weaning and dam-contact treatments were similar before separation. On the day of separation, gradual-wean cows spent more time with head over the perimeter fence compared with abrupt-wean cows ($t_{1.153} = 4.3; P < 0.001$), but weaning treatments were similar at 24 and 48 h after separation. Full-time cows spent more time with head over the perimeter fence (about 18 h/d), but full-time and part-time calves reduced lying time substantially on the day before separation, where part-time calves now had greater lying time than no-contact cows ($t_{1.172} = 2.7; P = 0.01$).

Daily 24-h lying time of calves was not affected by weaning treatment, but showed a 2-way interaction of contact treatment and day of separation (Figure 8b). Before separation, lying times were similar among contact treatments (about 18 h/d), but full-time and part-time calves reduced lying time substantially on the day of separation (to about 13.5 h/d; $t_{1.181} > 4.7; P < 0.001$); this reduced even further at 24 h after separation (to about 11.8 h/d; $t_{1.145} > 2.1; P = 0.03$), and had not returned to baseline levels at 48 h after separation (about 14.3 h/d; $t_{1.181} > 3.1; P < 0.01$). No-contact calves did not significantly change their daily lying time over the separation period (when only milk removal was experienced).

**Cumulative vocal response to weaning and separation**

For cows, the sum total (cumulative) vocalizations of any type over the weaning and separation observation periods did not differ between abrupt and gradual weaning treatments (134.4 ± 39.5 and 161.6 ± 45.1 vocalizations, respectively; $F_{1,43.4} = 0.20; P = 0.65$) or between full- and part-time contact treatments (143.8 ± 40.6 and 151.0 ± 42.9 vocalizations, respectively; $F_{1,43.2} = 0.02; P = 0.90$). For calves (excluding no-contact, which were only observed at separation), there was an interaction of weaning and contact treatment affecting
the sum total vocalizations of any type over the weaning and separation observations periods ($F_{1,46.7} = 5.4; P = 0.03$). Abrupt-weaned full-time calves vocalized the least compared with the other treatment combinations ($414.9 \pm 45.8$ vocalizations; $t_{1,47} > 2.9; P < 0.03$), with no differences among abrupt-weaned part-time, gradual-weaned full-time or gradual-weaned part-time calves ($736.0 \pm 90.8$, $649.1 \pm 71.0$ and $681.3 \pm 74.8$ vocalizations, respectively).

Figure 5. Least squares means frequency of vocalizations by dairy cows during the separation period, stratified by weaning treatment (abrupt or gradual) or dam-contact treatment (full-time or part-time). (A) High-pitched vocalizations; (B) Low-pitched vocalizations; (C) Mixed vocalizations. Vocalizations were recorded via direct observation during a 110 min period on the day before, day of, 24 h and 48 h relative to separation. Pairwise comparisons of a significant interaction (reported in Table 3) are indicated as significant ($P \leq 0.05$, indicated by *) or a tendency ($0.05 < P < 0.1$, indicated by †).
We expected that part-time cow-calf contact (suckling permitted during daytime only) and/or gradual weaning over 2 weeks (by reducing calf time with the dam) would reduce negative behavioral and vocal responses to separation in dairy cows and their calves. Our results revealed mixed support for this hypothesis, where behavioral responses mainly depended on the time since weaning and separation. Except for cumulative vocal responses in calves, there were no interactions between dam-contact and weaning treatments, so the effects of these treatments are discussed separately.

**DISCUSSION**

Figure 6. Least squares means frequency of vocalizations by dairy calves during the separation period, stratified by weaning treatment (abrupt or gradual) or dam-contact treatment (full-time or part-time). No-contact calves rarely vocalized and not shown. (A) High-pitched vocalizations; (B) Low-pitched vocalizations; (C) Mixed vocalizations. Vocalizations were recorded via direct observation during a 110 min period on the day before, day of, 24 h and 48 h relative to separation. Pairwise comparisons of a significant interaction (reported in Table 3) are indicated as significant ($P \leq 0.05$, indicated by *) or a tendency ($0.05 < P < 0.1$, indicated by $\dagger$).
Dam-contact duration: full-time or part-time?

To our knowledge this is the first study to examine how dairy cows respond to weaning and separation depending on daily contact duration with the calf. We found mixed support for the hypothesis that part-time contact reduces the negative behavioral responses to weaning and separation in either cows or calves.

Full- and part-time cows did not differ in vocal responses at gradual weaning initiation nor at separation, with the exception that part-time cows tended to continue high-pitch vocalizing at 48 h after separation. Cumulative vocalizations of cows over weaning and separation periods also did not differ among contact treatments. However, full-time cows showed stronger behavioral responses to separation during the observation periods (less lying time and more time with head over the perimeter fence), especially on the day of and 24 h after separation, compared with part-time cows. This was also supported by the daily 24-h lying activity, where full-time cows had a larger reduction in lying time compared with part-time cows on these days (although full-time cows had greater lying time than part-time and no-contact cows to begin with, both during the weaning period and day before separation, which may be related to differences in lying surface and/or space allowance; Tucker et al., 2022). It is possible that the bond between cow and calf was stronger in full-time than in part-time contact treatment, leading to more searching behaviors and less time lying at separation. On the other hand, frequency of vocalizations were similar between full- and part-time cows, so attempts

![Graphs showing behavior of dairy cows during separation](https://example.com/graphtest.png)

**Figure 7.** Least squares means of behavior of dairy cows during the separation period, stratified by weaning treatment (abrupt or gradual) or dam-contact treatment (full-time or part-time). (A) time spent lying; (B) time spent with head over the perimeter fence (back-transformed means and confidence limits). Behaviors were recorded via video recording during a 110 min period on the day before, day of, 24 h and 48 h relative to separation. Pairwise comparisons of a significant interaction (reported in Table 3) are indicated as significant ($P \leq 0.05$, indicated by *) or a tendency ($0.05 < P < 0.1$, indicated by $\lambda$).
to reunite through vocal communication appeared to be strong regardless of contact duration. Part-time cows experienced daily separations from their calves at night, so they may have learned that increased activity and searching behaviors while in the separation pen were futile at reuniting with their calves in another barn. Instead, part-time cows may have directed more efforts at vocal communication at separation, especially since auditory contact with calves was maintained. These explanations are speculative, but merits further exploration of how part-time cows respond to daily temporary separations, which may affect responses to permanent separation from their calves. For instance, there is some evidence that part-time cows (permitted night-time access to their calves while at pasture, compared with full-time access) were more restless at milking before anticipated reunion with their calves and groomed their calves more at reunion, suggestive of a rebound effect (Roadknight et al., 2022). These behaviors suggest part-time cows may experience repeated daily separations as negative; indeed, a judgement bias test at about one month after calving suggested part-time contact cows were in a negative emotional state compared with full-time cows (Neave et al., accepted). These studies and our current study suggest a need for improved understanding of how systems differing in daily duration of cow-calf contact may positively or negatively affect the cow (and calf, as described next).

For calves, it was hypothesized that part-time contact may ease the stress related to separation from the dam for several reasons: if calves are already accustomed to periods of separation from the dam (Weary et al., 2008; Veissier et al., 2013); if calves are consuming more solid feed due to restricted nursing opportunity (Bertelsen and Jensen, 2023); or if there is a weaker cow-calf bond that better prepares calves for independence from the dam. However, the vocal responses of calves at weaning and separation showed no clear effect of dam-contact duration. More part-time than full-time calves vocalized on the day of gradual weaning, but this reversed 24 h later when more full-time calves tended to low-pitch vocalize. Part-time calves also had the lowest daily 24-h

![Figure 8](image_url)  
Figure 8. Least squares means of daily 24-h lying behavior of dairy cows (A) and calves (B) during the separation period, stratified by weaning treatment (abrupt or gradual) or dam-contact treatment (full-time or part-time). Behaviors were recorded using leg-based accelerometers on the day before, day of, 24 h and 48 h relative to separation (recording interval from 1100 to 1100 h the next day, corresponding to time of separation). Pairwise comparisons of a significant interaction (reported in Table 3) are indicated as significant ($P \leq 0.05$, indicated by *) or a tendency ($0.05 < P < 0.1$, indicated by $\dagger$).
lying times. These vocal and lying patterns are likely related to hunger on the day of weaning initiation, since part-time calves were expecting to be reunited with the dams for nursing and were already experiencing at least 14 h since their previous milk meal. At separation, full-time calves tended to high-pitch vocalize more on the day of separation, perhaps as a response to a change of environment (Weary et al., 2008) and unexpected separation from the dam other than milking times. However, at 24 h after separation when the bulk of vocalizations occurred, part-time calves high-pitch vocalized substantially more than full-time calves (on average about 420 versus 260 vocalizations per calf, respectively). This result was unexpected, but may reflect a delayed ‘realization’ of permanent separation from the dam, given their familiarity with temporary separations during the night, and/or reflect an exaggerated response to hunger due to more than 24 h without milk and prior restricted suckling opportunity. Bertelsen and Jensen (2023) also found that behavioral responses of calves to weaning and separation were not reduced in part-time versus full-time contact systems, which were identical to the present system design. Notably, this was despite part-time calves spending more time eating solid feed during the night when dams were not present before weaning (Bertelsen and Jensen, 2023), but this may not have been sufficient to offset hunger at separation. Other studies have shown that calves will consume little solid feed even when suckling periods are short (Fröberg et al., 2008; Hepola et al., 2008; Roth et al., 2009). Low-pitch vocalizations occurred after separation, but at far less frequency than high-pitch vocalizations (about 5 per calf), which may reflect another means of coping when reunion through high-pitch calling is continuously futile (‘passive coping’ described by Siebert et al., 2011).

Overall, part-time contact did not appear to ease weaning and separation distress for cows or calves, for either of our weaning methods. However, other weaning strategies are known to mitigate separation distress, such as providing supplementary milk to calves after total separation from the dam (Johnsen et al., 2015a) so part-time dam contact may have beneficial interactive effects with alternative weaning strategies for the calves. We encourage future studies to identify practical ‘deboning’ strategies that successfully minimize separation stress in both cows and calves without compromising the benefits of cow-calf contact for the animals. For instance, other studies have manipulated the type of physical contact (e.g., calf restricted to an adjacent pen, without suckling, versus unrestricted access to the dam with suckling; Wenker et al., 2021, 2022) or level of nutritional dependency (e.g., calf with the dam only at night, can or cannot suck the dam, with or without supplementary milk; Johnsen et al., 2015a, 2018). Rather than imposing forced separations as done in our study, housing systems that encourage cows and calves to voluntarily spend time apart should be explored (Weary et al., 2008).

**Weaning method: gradual or abrupt?**

As expected, the initiation of gradual weaning induced a vocal response (primarily low-pitched) in both cows and calves, and gradual-wean cows responded over the next 24 h by spending more time close to and with their head over the calf creep fence, resulting in less time spent lying during the observation period. These behavioral responses are indicators of separation stress, interpreted as attempts to reunite with the calf (Flower and Weary, 2001a; Loberg et al., 2007; Stěhulová et al., 2008), and have been reported in previous studies using gradual weaning methods such as fence-line (Price et al., 2003; Enríquez et al., 2010; Johnsen et al., 2015b), and insertion of nose-flap to prevent the calf from suckling (Loberg et al., 2007; Ungerfeld et al., 2016). The increased frequency of these behaviors and low-pitched vocalizations were relatively short-lived, showing no differences by 48 h after weaning initiation, which were comparable to cows that experienced no change in calf contact (i.e., abrupt-wean treatment). Furthermore, daily 24-h lying time of cows was not impacted by gradual weaning initiation. Together, this suggests that cows may adapt to fence-line separation fairly quickly, as reported by others (Johnsen et al., 2015; Enríquez et al., 2010), or may show minimal reaction with regards to change in activity (Wenker et al., 2022). It is important to note that the fence-line weaning method commonly involves complete and sudden prevention of nursing, while our method permitted restricted periods of nursing. This approach may also have helped cows to adapt quickly to fence-line separation, once the cows learned that they are reunited with their calves for limited periods each day. The timing of weaning initiation likely also contributes to the strength of behavioral responses; gradual weaning initiation in our study began when calves were a minimum age of 8 weeks, which is far earlier than in nature when the calf is 7 to 14 mo (Reinhardt and Reinhardt, 1981). Thus, weaning initiation at later calf ages likely would help to limit or prevent negative behavioral responses at weaning; one study found that beef calves weaned at 180 d vocalized the least, where ‘extreme’ vocalizations were completely absent on the day of weaning, compared with calves weaned at 30 and 75 d (de Souza Teixeira et al., 2021). Future work should explore the effect of weaning age on the behavioral responses of dairy cows and calves, especially weaning after 3 mo of age given.
the paucity of research in dairy cattle for later weaning ages.

Contrary to our hypothesis, our gradual weaning method did not appear to better prepare the cows for complete separation from their calves. Rather, the bulk of cow behavioral responses during the observation period (reduced lying time; increased time with head over the perimeter fence) and vocal responses (frequency of all types of vocalizations) occurred on the day of separation rather than 24 h later, as seen in the abrupt-wean cows. Although at 48 h after separation there tended to be a reduced vocal response in the gradual-versus abrupt-wean cows, there was no overall positive effect of this gradual weaning method on reducing the cumulative vocal response of cows across weaning and separation observation periods. However, a positive result was in daily 24-h lying time, which suggested earlier recovery of lying time in gradual-wean cows at 24 and 48 h after separation. We had expected that progressively restricting daily contact between cow and calf over 2 weeks before permanent separation would assist in the ‘debonding’ process for both cow and calf. This may not have occurred because cows still had predictable reunions with their calves that permitted suckling. One study found that cows’ vocal response to fence-line separation from their calves was greater in cows that were able to nurse their calves compared with cows that were fitted with an udder net to prevent nursing (Johnsen et al., 2018), suggesting that nursing opportunity between dam and calf may be a contributing factor to high behavioral responses at complete separation. This could further explain why our gradual weaning method did not reduce behavioral responses at separation compared with abrupt weaning; reunion between cow and calf did not occur at the predicted time, so cows responded on the day of separation with a peak in high-pitched vocalizations and activity. This behavioral response is known to occur in free-ranging conditions when cows attempt to reunite with their calves after visual contact is lost (Padilla de la Torre et al., 2015). Perhaps permitting long periods of fence-line contact after milk removal may help to reduce vocal responses and prevent periods of lower lying time.

The pattern of vocalizations in calves after separation were similar to the cows, where gradual-weaned calves vocalized more the day of separation compared with abrupt-weaned calves. The bulk of vocalizations by calves occurred 24 h after separation for both gradual and abrupt weaning treatments, averaging over 300 high-pitched vocalizations per calf in just a single 110-min observation period. Daily 24-h lying time also reduced after separation in both abrupt- and gradual-weaned calves. This high frequency of vocalizations and change in lying time by calves is likely in part related to hunger due to time since a previous milk meal; on the day of separation, gradual weaned calves were unable to nurse the dam so these calves had already experienced at least 20 to 22 h since their previous milk meal compared with abrupt-weaned calves, who could have nursed up until the time of separation. Then, after 24 h with no milk meal, the abrupt-weaned calves responded with a peak in vocalizations. Previous work has indicated that providing milk to calves after separation from the dam can reduce the vocal response (Thomas et al., 2001; Johnsen et al., 2018), suggesting that milk removal is a contributor to the stress responses at separation. Hunger can result if calves are not sufficiently eating solid feed at the time of milk removal (Borderas et al., 2009). Because weaning in commercial systems occurs earlier than in nature, gradual weaning from milk is a common method to encourage calves that are artificially fed milk to begin consuming solid feed before milk is completely removed (Khan et al., 2011). Our companion study (Jensen et al., in press) found that our gradual weaning method reduced nursing duration, but possibly not milk intake, so calves likely were not consuming much solid feed at the time of separation. This issue has been raised in previous studies of calves weaned in cow-calf contact systems (Fröberg and Lidfors, 2009; Fröberg et al., 2011). In future, gradual weaning methods for cow-calf contact systems should be designed with the goal to successfully transition calves onto a fully solid feed diet and include solid feed intake as an outcome measure. One possibility is to have a cow-driven system where nursing events are decided by the cow while the calf is restricted to a specific area (Johnsen et al., 2021), or to wean at much later ages when calves are less reliant on milk.

The high vocalization frequency by calves at separation could also be related to the loss of social contact with the dam. If the vocalization response to separation was purely related to hunger due to milk loss, a similar vocalization response and change in lying behavior should be seen in calves weaned in the same manner but reared without the dam. However, our no-contact calves rarely vocalized and showed little change in lying time after milk intake ceased, regardless of whether this was done abruptly or gradually over 2 weeks. This suggests that the behavioral response to separation in dam-reared calves is also related to the cow-calf bond, which develops between 1 and 4 d after birth (Weary and Chua, 2000; Flower and Weary, 2001b; Stěhulová et al., 2008). Bonded animals show reinstatement behaviors upon separation, such as vocalizations that function to facilitate reunion between mother and offspring (Newberry and Swanson, 2008), or seeking behaviors, such as time with head out of the pen, that suggest a
Interactive effects of dam-contact duration and weaning method

The only interactive effect of dam-contact duration and weaning method for any of our measures was for cumulative vocalizations (over weaning and separation periods) in calves. We had predicted that part-time dam-contact combined with gradual weaning could reduce the behavioral response to weaning and separation the most. However, it was abrupt-weaned full-time calves that vocalized the least (about 400 vocalizations per calf) compared with other treatment combinations (about 700 vocalizations per calf), excluding no-contact calves who rarely vocalized at the time of complete milk removal (1 vocalization per calf). This pattern was not observed in the cows (similar across treatment combinations, about 150 cumulative vocalizations per cow). Again, the high rate of vocalizations in calves is probably related to hunger upon milk deprivation (Thomas et al., 2001), where gradual-weaned or part-time calves were likely more hungry on the day of weaning and separation due to restricted suckling opportunity (driving up vocalization frequency), while abrupt weaned calves experienced no change during the weaning period (minimizing vocalization frequency). A study in fence-line weaned beef calves also found the overall response to weaning and separation was higher than abrupt-weaned calves, which the authors suggested to be due to the prolonging of weaning via fence-line (Enriquez et al., 2010).

Another possible explanation is that all treatments except the abrupt-weaned full-time calves had some type of expectation regarding reunion with the dam, and the permanent separation and timing went against these pre-formed expectations. When animals perceive a mis-match of expectations with reality this can lead to negative emotional states (Eldar et al., 2016; Raoult et al., 2017), expressed in the form of vocalizations which may be exaggerated in the context of cow-calf separation (Green et al., 2021; Schnaider et al., 2022). This result should not be taken that abrupt weaning methods are the way forward; there is other evidence to the contrary that stepwise or 2-step methods are beneficial in dairy calves (Bertelsen and Jensen, 2023; Johnsen et al., 2015b; c) and beef calves (Price et al., 2003; Haley et al., 2005; Taylor et al., 2020). Rather it brings to light that the prior expectations of cow and calf are likely important mediating factors affecting how animals respond to unexpected situations; these should be considered when evaluating new cow-calf management and weaning strategies.

Limitations and further considerations

Our results are dependent upon the timing of our observation period which was in the 2 h window immediately after weaning and separation, and every 24 h thereafter for 2 d (but not at the same time of day: 0530 h for weaning, 1100 h for separation). Graphical examination of the distribution of vocalizations over our observation days after separation shows that high-pitch vocalizations generally return close to baseline around 48 h, but low-pitch and mixed vocalizations remain somewhat elevated for both cows and calves. This suggests that observation beyond 48 h may better capture the extent of weaning and separation distress. Additionally, observation periods longer than 2 h per day may capture delayed vocal responses to hunger in calves. The vocalization rate of control calves may have been greater if the observation period was around...
expected feeding time (0630 and 1700 h rather than midday at 1100 h), as observed by Bertelsen and Jensen (2023).

Calves were moved out of their home pen to a novel pen at the time of separation, and could hear vocalizations of their dam and other cows; both of these conditions could have contributed to additional stress leading to an exaggerated vocal response. An alternative approach could be to remove the cows from the pen rather than the calves, since cows were familiar with leaving their pens already. Additionally, calves may have vocalized more in response to the presence of the human observers, although only no-contact calves had a direct association between humans and milk delivery.

Some complementary measures would be useful for improving interpretation of weaning and separation responses depending on our dam-contact and weaning treatments. For instance, milk yields and nursing duration in full-time and part-time cows before and during the time of weaning could reveal if our gradual weaning method was (approximately) reducing milk intake by calves. Furthermore, measures of the strength of the bond between dam and calf would be useful for understanding if vocal responses to separation are more related to the strength of bond, hunger or both. Such information would help to refine what elements of the weaning process should be targeted to reduce distress around weaning and separation as much as possible.

CONCLUSIONS

Overall, part-time contact between dam and calf (daytime contact only, compared with full-time) or gradual weaning (progressive reduction in contact between dam and calf, compared with abrupt cessation of milk and dam contact) did not reduce the behavioral responses of cows and calves to weaning and separation. This suggests that the design of the part-time contact and gradual weaning methods probably resulted in hunger that was expressed earlier after intervention, and did not adequately contribute to diminishing the bonding between cow and calf. In addition, the expectation for reunion may have exaggerated behavioral responses at separation. Regardless of whether weaning and separation are done at once (i.e., abruptly), gradually (i.e., milk and dam removal occur over time) or in steps (first milk removal, then dam removal, or vice versa), the transition involves several factors that contribute to distress at this time, such as changes in nutritional, social and physical environments. Future research should explore methods that gradually reduce milk intake (thus stimulating solid feed intake) and gradually diminish the maternal bond (promoting social and emotional independence).

ACKNOWLEDGMENTS

We gratefully thank the barn staff at the Danish Cattle Research Centre, Aarhus University (Denmark) for the management and care of all cows, and to all the students for their assistance during barn and/or video observations: Vanessa De Jesus and Clara Osmond (AgroSup Dijon, France), Martin Clipet (Institut Polytechnique UniLaSalle, France), Ze Yin, Astrid Schroll, Maja Bertelsen, and Allison Welk (Aarhus University, Denmark). We are also grateful to technicians Carsten Christensen, John Misa Obidah, and Henrik Andersen (Aarhus University, Denmark) for construction and assistance with various project elements. Thank you also to Maarit Mæupiä for statistical consultation. Study funding was provided by Independent Research Fund Denmark (Odense, Denmark), and is part of a larger research project investigating the potential welfare benefits of cow-calf contact for dairy cows (Can dairy cows have the best of both worlds?; 2020-2024).

REFERENCES


ORCIDs

Heather W. Neave https://orcid.org/0000-0002-1818-8131
Emma Hvidtfeldt Jensen https://orcid.org/0000-0002-1478-2870
Marine Durrenwachter https://orcid.org/0009-0002-5075-9261
Margit Bak Jensen https://orcid.org/0000-0001-7982-582X