2-14-2019

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An Exploration of Infant and Toddler Unstructured Outdoor Play

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Abstract

Unstructured outdoor play is important for children's development. The present study examined infants' and toddlers' physical and social unstructured outdoor play behaviors within childcare centers. Children's outdoor play behaviors were video recorded at two centers (A&B) and assessed using a modified version of the Observational System for Recording Physical Activity in Children in Preschool (OSRAC-P). Children in this study primarily took part in active play (56.7%) and engaged in play without any type of prompting from a teacher (91.2%). There was a significant interaction between the effect of center and location on physically active play ($p<0.001$). Children at Center B were most active in open play areas (77.6%), while children at Center A were most active in gross motor play areas (72.2%). In sum, the outdoor play environment influences infants' and toddlers' physical and social play behaviors; however, more research is needed to determine the optimal environment for development.

Keywords: Play, Infant, Toddler, Childcare, Outdoor, Activity

Introduction

Providing opportunities for play is a crucial component of the overall health and well-being of young children. Unfortunately, young children are spending a concerning amount of time in sedentary behaviors (Lauricella, Wartella, and Rideout 2015; Prioreschi et al. 2017). For example, a study by Prioreschi et al. (2017) found 3-month old infants were already watching 30 minutes of television per day and spending over 2 hours per day in restrained activities (e.g., car seat). Evidence suggests that increased time spent in sedentary behaviors such as television viewing is associated with increased adiposity as well as decreased psychosocial health and cognitive development in children less than 4 years of age (LeBlanc et al. 2012). Thus, it is imperative to reduce sedentary behaviors to promote proper physical, psychosocial, and cognitive development. One way to accomplish this is by providing opportunities for active play (Clements 2004). For example, play allows children to develop physically, by promoting gross and fine motor skills; socially, by supporting peer interaction; and cognitively, by allowing children to use problem solving and investigation skills (Clements 2004; Ginsburg 2007).

While promotion of all play types are important, there are meaningful benefits to children's engagement in unstructured outdoor play. Unstructured outdoor play consists of open-ended play that allows freedom to construct rules, goals, and meaning (De Valk, Kekker, and Eggen 2013). Unstructured outdoor play provides children opportunities to create their own sensory experiences in various play environments, such as playing in a

sandbox or running in the grass. Outdoor unstructured play also provides opportunities for improvement of physical and social competencies by playing with peers on a variety of surfaces and structures such as grass, sand, and slides (Iioven et al. 2016; Pellegrini 1992). It supports children's autonomy and imagination, as children get to select how and with what they play (Clements 2004). It can also support overall health as pre-schoolers (3-5 year olds), in outdoor play environments, when compared to indoor environments, have been shown to take part in more vigorous physical activity (Iioven et al. 2016). Due to many of these benefits, there has been a call for more unstructured outdoor play time in lieu of more passive forms of entertainment such as listening to stories (Murray et al. 2013). Despite this evidence, additional research is needed to explore how to support children's engagement in unstructured outdoor play.

One setting that has the potential to offer unstructured outdoor play opportunities on a regular basis is formal childcare (Story, Kephingst, and French 2006). Reports of the percentage of children in formal childcare in developed countries range from 20-39% (Eurostat 2018; Laughlin 2013). Thus, childcare facilities are prime locations to promote play.

Exploration of outdoor play environments within childcare settings has determined the vast majority of outdoor play spaces offer a multitude of play opportunities via playground structures, open surfaces, and loose parts (e.g., cardboard boxes, fabric) (Acar 2014; Dowda et al. 2009; Olsen and Smith 2017). However, minimal research has been done

to examine how children utilize these play opportunities. The research that has been done has primarily focused on preschoolers' (3-5 years) social and physical play behaviors (Gubbels, Van Kann, and Jansen 2012; Li and Wang 2016). Research examining social play behaviors determined pre-schoolers primarily engage in high amounts of pretend play behaviors (Li and Wang 2016). The physical play line of inquiry has shown directly observed physical activity was positively associated with the availability of portable jumping equipment and an outdoor track, and negatively associated with portable slides, swinging equipment, and sandboxes (Gubbels et al. 2012). In addition, four-year old children appeared to engage in greater amounts of activity when engaging in solitary play versus peer group play or adult-led play (Iioven et al. 2016). This information can assist providers in selecting equipment to promote physically active play. However, little research has explored the unstructured outdoor play behaviors of children three years of age and younger. It is crucial to understand how infants (0-1 year of age) and toddlers (1-2 years of age) engage in outdoor play, as children of this age are undergoing fundamental physical and social development (UNICEF 2014). Therefore, the purpose of this study was to determine infants' and toddlers' physical and social unstructured outdoor play behaviors within childcare.

Methods

This observational cross-sectional study examined the unstructured outdoor play of infants and toddlers (age 0-3 years) at two childcare centers – licensed childcare providers that typically offer care in larger groups with multiple staff providers. This study was approved by an Institutional Review Board and conducted in the spring of 2017.

Setting and Participants

The childcare centers were located in a large metropolitan city within the Midwestern United States. The centers were recruited to participate through existing contacts of the lead investigator. Centers were chosen in order to represent diverse characteristics and teaching philosophies. Center A was a Montessori site primarily serving Caucasian and middle-to-high income families. The size of the classroom ranged from 20-24 children with one lead teacher and four assistants. Center A had two toddler classrooms and one infant classroom. Center B was an Early Head Start center-based site that served minority families - primarily African American - and low-income families. The size of the classroom consisted of no more than eight children with one lead teacher and two assistants. Center B had four toddler and four infant classrooms. The ages of children in the infant classrooms typically ranged from 6 weeks-18 months, while the age of children in the toddler classrooms were 18-36 months. Data were collected during March and April of 2017 and the average temperature was 9.86 degrees Celsius (range 2.7-22.7°C).

The lead investigator contacted the executive director of each center to explain the study. Once the executive directors agreed to participate, they discussed the study with their teachers and provided the contact information of teachers who agreed to participate to the research team. The research team then met with the teachers and assistants to explain the study and complete the consent form. To obtain parental permission for the video observation, parents were first informed about the study by researchers at a parent's night event and/or in a short e-mail developed by the researchers that was sent to parents by teachers. Once parents were notified, the executive directors and lead teachers disseminated and collected the parental consent forms. Children were included in the study if their parents returned a signed consent form. Any child whose parent did not complete the parental consent form or staff who did not want to participate were provided a separate play area

outside of the viewing area during data collection. Overall, 49 parents completed consent forms for their child (n=18 infants, n=31 toddlers).

Measures

A modified version of the Observational System for Recording Physical Activity in Children in Preschool (OSRAC-P) was utilized. The OSRAC-P has demonstrated the ability to provide contextually rich information regarding children's play behaviors; and observations have previously been undertaken with children as young as two years of age (Gubbels et al. 2012). Further utilization of this tool within infant and toddler populations could allow childcare providers and researchers to determine what factors best support unstructured outdoor play opportunities. Since physical and social outdoor play were the focus of the study, only the following categories from OSRAC-P were utilized: physical activity level, activity type, play context, initiator of activity, group composition, and prompt for activity (Brown et al. 2006). Physical activity level codes (n=6) were modified from the Children's Activity Rating Scale to indicate sedentary (stationary + stationary with limb movement), light (slow, easy movement), moderate to vigorous (moderate activity and vigorous activity) and active (slow, easy movement, moderate activity, vigorous activity) behaviors (Pate et al. 2008; Puhl et al. 1990). Activity type codes (n=19) consisted of identifying specific types of movement such as climbing, running, sitting, squatting, and standing among others. Play context codes (n=15) focused on identifying usage of outdoor play surroundings (e.g., balls, fixed equipment, open space). Initiator of activity codes (n=3) consisted of identifying who initiated the play activity (adult, child, or peer). Group composition codes (n=6) identified whether a child was alone or with an adult or peer in a group. Prompt for activity codes (n=6) determined whether a teacher or peer promoted increases or decreases in active play.

In addition to the categories outlined by OSRAC-P, a play area category was added to identify and differentiate between general play areas of infants and toddlers within the outdoor play spaces. The play areas were divided into three distinct locations: open space, gross motor play area, and fine motor play area. The areas were identified based on the primary function of the equipment within that area. Open spaces were defined as green spaces and sidewalks. Gross motor play areas were defined as areas with large play structures that allowed for gross motor movements such as running or walking. Fine motor play areas were categorized as areas with small equipment (e.g., pails, shovels) that allowed for fine motor skills like grasping or pulling. At Center A, approximately 94.1% of the play area was categorized as open space, 4.8% gross motor play area, and 1.1% fine motor play area within both the 804.6 sq. meters infant play area and the 1839.2 sq. meters toddler play area. At Center B, approximately 82.1% of the play area was categorized as open space, 11.2% gross motor play area, and 6.7% fine motor play area within the 430.7 sq. meters play area.

Figure 1. Overview of Center A

Figure 2. Sample of Center A Infant and Toddler Areas

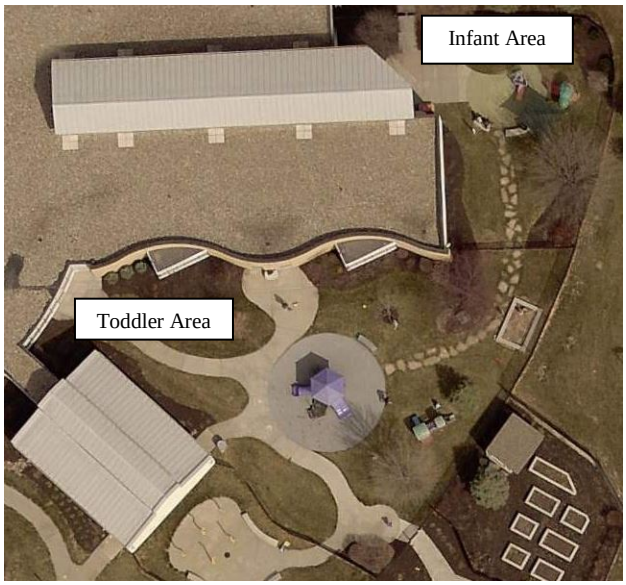

Figure 3. Overview of Center B

Figure 4. Sample Center B Infant Area

Figure 5. Sample of Center B Toddler Area

Protocol

Children and staff were observed through video recordings during their typical unstructured outdoor play time once per day for five days. Classroom schedules at and between each site varied indicating a range of a total of 25-90 minutes of scheduled outdoor

play time one to two times a day. Data were recorded during a morning unstructured outdoor play time if more than one time was offered. For each data collection session, three iPads were set up on movable tripods throughout the space that the class primarily used. At least two research personnel were at each site for every data collection. Research personnel were instructed that if there were no children in the play area, they could move the tripod to find the nearest child not currently being videotaped. A fourth iPad was available, as needed, to capture additional child movement outside of the other areas being video recorded. Recordings began as soon as a child entered the outdoor play area and ended when the last child left the outdoor area. Length of recordings for both centers ranged from 15-40 minutes, with an average of 26 minutes.

Data Analysis

To analyze the data, the lead author, experienced in direct observation, conducted training for three observers on the OSRAC-P. The training consisted of reviewing the OSRAC-P manual, achieving a score of 90% or above on a 45-question test about the manual developed by the lead author, as well as group and individual practice with the lead author. Inter-observer reliability checks were completed on 10% of observations intermittently throughout data analysis. Average kappa scores ranged from .96-.98, indicating excellent inter-observer reliability. Kappa scores for each categorical variable were as follows: outdoor play context - .96, physical activity level - .97, physical activity type - .96, group composition - .98, initiator of activity - .98, prompt for physical activity - .98. All categories and responses were entered into an Excel document. Each observation interval consisted of observing the child for five seconds with 25 seconds for recording within the Excel document. Overall, 3,007 observations were completed of Center A and 3,039 observations were completed of Center B.

Descriptive statistics were first computed to evaluate combined physical activity level, activity type, play context, initiator of activity, group composition, and prompt for activity for both sites. An additional analysis was conducted to compare activity level and type between centers. A 2-way ANOVAs was used to examine the effect of center and play area location, and center and play type.

Results

When assessing OSRAC-P findings overall, a few key results emerged. In regard to physical activity level, infant and toddler children most often participated in active movement (56.7%) or sedentary behaviors (41.9%). Children were most frequently engaging in walking (27.6%) or standing (20.5%). When evaluating the context of play, children primarily played in open space areas (43.2%) and fixed playground areas (37.6%). The majority of the time children were initating play activities (91.3%) rather than having play initiated by an adult; however, children were most frequently found playing with an adult present (49.0%) or by themselves (20.8%). Further, the majority of the time children engaged in play without any type of prompting from a teacher (91.2%). Less than 1.5% of the time researchers were unable to record a behavior or activity as displayed by the “can’t tell” variable seen in Table 1.

Table 1.Combined frequencies of observed behaviours for Centers A and B

Physical Activity Level	*n*	%
Sedentary	2532	41.9
Light	2045	33.8
Moderate	1385	22.9
Active	3430	56.7
Can't Tell	84	1.4
Physical Activity Type		
Climb	288	4.8
Crawl	190	3.1
Dance	5	0.1
Jump/Skip	48	0.8
Lie Down	37	0.6
Pull/Push	164	2.7
R & T - Rough and Tumble	17	0.3
Rock	41	0.7
Run	785	13.0
Sit/Squat	1152	19.1

Stand	1242	20.5
Swing	243	4.0
Throw	76	1.3
Walk	1667	27.6
Other	6	0.1
Can't Tell	85	1.3
Outdoor Play Context		
Ball	334	5.5
Fixed	2273	37.6
Game	5	0.1
Open Space	2611	43.2
Portable	352	5.8
Sandbox	30	0.5
SocioProps	249	4.1
Wheel	154	2.5
Other	4	0.1
n/a	17	0.3
Can't Tell	17	0.3
Initiator of Activity		
Adult	457	7.6
Child	5517	91.3
Can't Tell	72	1.1
Group Composition		
Solitary	1259	20.8
1-1 Adult	441	7.3
1-1 Peer	381	6.3
Group Adult	2964	49.0
Group Peer	951	15.7
Can't Tell	50	0.8
Prompt for Activity		
None	5515	91.2
Teacher Prompt to Increase	307	5.1
Teacher Prompt to Decrease	107	1.8
Peer Prompt to Increase	35	0.6
Peer Prompt to Decrease	8	0.1
Can't Tell	74	1.2

A few key differences were apparent when comparing Center A and Center B. There was a significant interaction between the effects of center and location on physically active play $F(2, 5267)=81.41$, $p<0.001$. Center B children were more active in open play areas (77.6%) than children in Center A (57.4%). Children at Center A achieved the most active time in the gross motor play areas (72.2%) and the least active time in fine motor play areas (45.3%). Further, moderate to vigorous physical activity was highest within gross motor play areas (28.3%) and sedentary activity was greatest in fine motor play areas at Center A (51.4%). When examining Center B, children were most active in the open play areas

(77.6%) and also least active in the fine motor play areas (33.9%). Center B children were also most likely to be sedentary when located in the fine motor play areas (66.0%).

Table 2. Percentage of time spent in activity in relation to play area

	Center A				Center B			
Location	Sedentary	Light	MVPA*	Active	Sedentary	Light	MVPA*	Active
Open Play Area	42.3	29.4	28.0	57.4	22.2	46.8	30.8	77.6
Gross Motor Play Area	23.4	43.9	28.3	72.2	33.2	33.1	30.1	63.2
Fine Motor Play Area	51.4	38.2	7.1	45.3	66.0	24.9	9.0	33.9

** MVPA=Moderate to Vigorous Physical Activity*

***Percentages do not equal 100 due to combined physical activity level data.*

Table 3. Percentage of time spent in types of physical activity by center

PA Type	Center A (%)	Center B (%)
Climb	2.6	7.0
Crawl	1.7	4.6
Dance	0.0	0.1
Jump/Skip	0.7	1.0
Lie Down	0.2	1.1
Pull/Push	1.9	3.6
Rough & Tumble	0.3	0.3
Rock	0.1	1.3
Run	12.2	13.8
Sit/Squat	20.5	17.6
Stand	17.2	23.9
Swing	8.1	0
Throw	1.0	1.6
Walk	32.7	22.5
Other	0.8	1.6

Significant differences were also seen between center and activity type, $F(2, 784)=109.65$, $p<0.001$. Specifically, children at Center A were most frequently walking (32.7%) or swinging (8.1%), whereas children at Center B more often took part in standing (23.9%), climbing (6.9%), or crawling on equipment (4.5%; Table 4). When evaluating children's behaviors based on location, significant differences were also seen, $F(2,$

2503)=64.03, $p<0.001$. At both sites children engaged in running (Center A = 21.69%, Center B = 21.47%), walking (Center A = 48.77%, Center B = 22.99%), and standing behaviors (Center A = 14.61%, Center B = 27.97%) most often in open space areas, and in sitting/squatting behaviors in fine motor areas (Center A = 40.43%, Center B = 24.38%). Additional findings can be found in Table 4.

Table 4. Percentage of time spent in play area

	Center A			Center B		
PA Type	Open Area	Gross Motor	Fine Motor	Open Area	Gross Motor	Fine Motor
Climb	1.0	6.2	2.8	2.1	25.8	14.1
Crawl	1.1	0.7	2.9	2.4	1.6	14.3
Dance	0.1	0.0	0.0	0.3	0.0	0.0
Jump/Skip	1.1	0.2	0.3	1.0	1.2	0.9
Lie Down	0.1	0.5	0.1	0.2	7.4	1.1
Pull/Push	2.8	2.6	0.2	2.4	2.5	1.2
Rough & Tumble	0.4	0.2	0.2	0.0	0.0	0.0
Rock	0.1	0.0	0.0	1.8	0.0	2.1
Run	21.7	1.3	5.0	21.5	2.9	1.6
Sit/Squat	6.5	17.9	40.4	13.8	20.9	24.4
Stand	14.6	9.2	24.7	29.0	7.4	17.5
Swing	0.2	44.9	0.0	0.0	0.0	0.0
Throw	1.4	0.0	0.9	2.3	0.0	0.4
Walk	47.8	10.7	22.4	23.0	26.2	19.4
Other	1.0	0.0	0.0	0.1	0.0	0.0
Can't Tell	0.1	5.6	0.1	0.1	4.1	3.0

Discussion

The purpose of this study was to determine infants' and toddlers' physical and social unstructured outdoor play behaviors within childcare. The findings from the present study provide key information regarding the unstructured outdoor play behaviors of infants and toddlers that can be utilized for future practice and research. Important findings regarding physical and social play are discussed in more detail below.

Overall, children took part in physically active play for a little over half of the time they were outdoors. Current best practice in childcare recommends daily outdoor play time

for both infants and toddlers (Caring for our Children 2018). Specifically, recommendations suggest infants be taken outside 2-3 times per day and toddlers be offered 60-90 minutes of outdoor play daily. Practices in both centers did not always align with these best practices. For example, not all infants were taken outside multiple times a day and toddlers were not always offered 60+ minutes of outdoor time. To ensure children are receiving all of the benefits associated with outdoor unstructured outdoor play, efforts are need to help staff schedule outdoor time. Additionally, future research studies are needed to examine the amount of unstructured outdoor play provided, along with the amount of physically active play accumulated in relation to a child's physical health (e.g., weight), mental health, and cognitive health. Understanding this connection, could further motivate teachers to meet best practices for outdoor time.

Consistent with research in older children, open play areas and gross motor play areas were associated with higher amounts of physically active play (Nicaise, Kahan, and Sallis 2011; Nicaise et al. 2012; Trost, Ward, and Senso 2010). Thus, to allow for active play and subsequent health benefits, educators should consider offering open play and gross motor play areas if possible. Interestingly, children at Center B were more physically active in open play areas compared to Center A. This finding was especially interesting given Center A had larger amounts of open play areas. There are several possible explanations for this finding. One explanation could be related to the characteristics of the children (e.g., race/ethnicity, weight) involved in the study, characteristics or behaviors of the caregivers (e.g., engagement with children outside of outdoor play time), or that there might be an optimal amount of open space that promotes active play. However, more research is needed specific to infants and toddlers to provide best practice recommendations regarding these items.

Alternatively, this finding could be due to the availability of portable play equipment in these spaces such as balls or hula hoops as other research suggests the availability and

quality of portable play equipment can greatly influence active play (Gubbels et al. 2012; Trost, Ward, and Senso 2010). While not reported in this paper, we did examine but found no significant differences in the amount of portable play equipment provided. However, providers should be encouraged to provide enough quality portable play equipment to encourage active play. Another potential explanation for this finding is in regard to the characteristics of the space (i.e., surface, proximity to other play areas, obstructions). For example, at Center A the open space was primarily on a hill in the corner of the play space whereas at Center B the open space was flat and more centrally located. Children may have been interested in being closer to other play structures, teachers, or peers at Center B due to the central location. Future development of outdoor play areas could also consider offering open play spaces of various grading levels. A space with flat areas may be more easy to navigate for those mastering gross motor ability but hills could also challenge and improve the motor skills of others.

While children were less likely to be active in fine motor areas, these areas may provide opportunities to develop other important physical skills such as fine motor skills and social skills such as relationship building. Interestingly, research in landscape design suggests that playgrounds with a more nature-based design not only facilitate physical and social development, but also promote fine motor development, creative play, and sensory stimulation (Woolley and Lowe 2013). Thus, while it is important to encourage active play, other areas of development should not be neglected and playground environments should be designed to encourage multiple areas of development for children.

Additional differences were found between the centers and several of these findings may be due to the type of fixed playground equipment that was available. For example, children at Center A were more frequently swinging – this behaviour was not prevalent at Center B because this site did not have swings available. Further, while both sites had a

playground structure, at Center B this was the focal point of the play areas while at Center A there were other areas of fixed equipment and open play spaces. This could explain why children at Center B were more often climbing than children at Center A. Importantly children at Center A were most frequently walking while children at Center B were more frequently standing. More research is needed on influential sociodemographic or additional environmental factors for infant and toddler outdoor play experiences. Unfortunately, this study was unable to obtain this data in order to understand if this influenced our findings. Future research should consider comparing similar play environments to determine the role of the environment or sociodemographic variables in children's unstructured outdoor play behaviors.

In regard to social play, children were the primary initiators of play and there were limited observations of teachers prompting activity. It should be noted that in personal conversations with teachers, it was mentioned that they are encouraged to allow children to lead play activities during unstructured outdoor playtime. Thus, this finding likely represents a pedagogical practice of both centers. Specifically, Montessori trained educators are often encouraged to promote free play with natural play materials (Saracho and Spodek 1995). In addition, Early Head Start program teachers are encouraged to support children's self-awareness through exploration and environment manipulation. They are also encouraged to support the use of natural play materials (Early Head Start National Resource Center 2013). Despite the obvious benefits of supporting autonomous free play, research in childcare centers for preschool-aged children found staff behavior significantly influences children's behaviors (Bell et al. 2015; Trost et al. 2010; Vanderloo et al. 2014). Specifically, when staff prompt children to be active and join in on active play, children's participation in physically active play can improve (Trost et al. 2010). Thus, future efforts should continue to encourage

infant and toddler staff to actively promote children's participation in active play opportunities.

There were several limitations to this study. First, observations were coded via video recordings resulting in occasional periods in which the observers' view was obstructed or the child was out of the field of view and they were unable to see the observed child. Thus, optimal coding of playground behaviors was not always possible. Additionally, although audio was recorded, it was challenging to hear teachers if they were far from the camera, had their back to the camera, or if other noise was present (e.g., wind, children playing). This issue made it challenging to accurately code verbal prompts by teachers and children. Future research could examine differences between video observation and in-person coding of the OSRAC-P (Loprinzi and Cardinal 2011). Second, the OSRAC-P has not been validated with infants and toddlers (ages 0-3). Given that a similar tool is not available, the authors believed this was still a viable tool for describing young children's play; however, results should be interpreted with caution until further studies can validate these findings. Lastly, there was variance in weather while video data was being recorded. At both locations, because recordings did not occur simultaneously at each center, there may have been a significant difference in the outdoor conditions on the days when data were recorded which could have impacted the behaviors of teacher and children, making it difficult to compare between centers. For example, on a colder day, some teachers may have prompted more active play compared with a warmer day when activities like sandbox or chalk play might have been initiated.

This study is strengthened by using an observational system with over 6,000 observations to describe infants and toddlers physical and social play in an unstructured outdoor play environment within childcare. Additionally, this study was one of few to utilize the OSCRAC-P with infants and toddlers. Although the OSCRAC-P has not been validated

for use in young children, after completing data collection and analysis the research team felt as if this tool was still able to provide contextually rich information regarding children's play behaviors. Specifically, the revised coding framework of the OSCRAC-P contained the necessary content to code all of the play behaviors observed in infants and toddlers. In order to promote optimal outdoor play environments, it is necessary to first understand how children play and interact outdoors. Thus, this study provides a foundation for future research studies. An additional strength regarding this study is that it employed a vastly diverse sample in regard to the participants and childcare locations.

Conclusion

Although unstructured outdoor play is essential to the growth and development of children, little is known about the outdoor play behaviors of children less than three years old. The present study used direct observation to examine infants' and toddlers' outdoor play behaviors at two childcare centers. Findings indicate the environment of outdoor play areas influences infants' and toddlers' behaviors. By offering time, an open play space, and staff who promote active play, infants and toddlers could accumulate more physically active play. These results can help guide researchers and practitioners aiming to understand and/or improve the development of children as well as their outdoor environment. Additional research is needed to continue to explore the impact staff promotion efforts, sociodemographic variables, and environmental factors have on infant and toddlers unstructured outdoor play. Further, it is important to think of the development of outdoor play environments as a process. When trying to create an optimal outdoor play environment it is crucial to not only consider the optimal amount of outdoor space but all impacted entities including children, families, educators, administration, and any outdoor play regulations (Olsen and Smith 2017).

Funding

This work was supported by the University of Nebraska Foundation.

Disclosure Statement

The authors have no conflicts of interest to report.

Data Availability

The data set can be made available by contacting the corresponding author.

Compliance with Ethical Standards

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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